

FORM PTO-1390 (REV. 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			REG 670A-US
			U.S. APPLICATION NO. (if known, see 37 CFR 1.5 Not Yet Known 097868677
INTERNATIONAL APPLICATION NO. PCT/US99/30900	INTERNATIONAL FILING DATE 23 December 1999	PRIORITY DATE CLAIMED 23 December 1998	
TITLE OF INVENTION METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS			
APPLICANT(S) FOR DO/EO/US Samuel J. Davis, Nicholas W. Gale, George D. Yancopoulos, Neil Stahl			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. 4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto. b. <input checked="" type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)). 9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 			
Items 11 to 20 below concern document(s) or information included:			
<ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. 14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 15. <input type="checkbox"/> A substitute specification. 16. <input type="checkbox"/> A change of power of attorney and/or address letter. 17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 20. <input checked="" type="checkbox"/> Other items or information: An unexecuted Declaration and Power of Attorney 			

U.S. PATENT NO. (if known, see 37 CFR 1.5) 097/868877		INTERNATIONAL APPLICATION NO. PCT/US99/30900		ATTORNEY'S DOCKET NUMBER REG 670A-US	
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21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY <div style="display: flex; justify-content: space-between;"> \$ 710. </div>	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).					
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	77 - 20 =	57	x \$18.00	\$ 1,026.	
Independent claims	6 - 3 =	3	x \$80.00	\$ 240.	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ \$270.00	
TOTAL OF ABOVE CALCULATIONS =				\$ 2,246.	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				+	
SUBTOTAL =				\$ 2,246.	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).					
TOTAL NATIONAL FEE =				\$ 2,246.	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +					
TOTAL FEES ENCLOSED =				\$ 2,246.	
				Amount to be refunded:	\$
				charged:	\$

a. ☒ A check in the amount of \$ 2,246. to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees.
 A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
 overpayment to Deposit Account No. 18-0650. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card**
 information should not be included on this form. Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR
 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
 Linda O. Palladino
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 777 Old Saw Mill River Road
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Linda O. Palladino
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45,636
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Att. Dkt. No. - REG 670A-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Davis, S., et al.

U.S. Serial No.: Not yet known

Examiner: Not yet known

Filing Date: Filed herewith

Group Art Unit: Not yet known

Title: METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY
OF LIGANDS

June 20, 2001

Commissioner of Patents and Trademarks
United States Patent and Trademark Office
Washington, DC 20231

SIR:

PRELIMINARY AMENDMENT

This paper is submitted in connection with the above-identified application, which is a U.S. National Stage Application of PCT International Application No. PCT/US99/30900, filed December 23, 1999, which claims priority to U.S. Provisional Application No. 60/113,387, filed December 23, 1998, now abandoned. Prior to examination of the application on the merits, Applicants respectfully request entry of the following amendments to the claims and specification:

In the claims:

Please cancel claims 1-41 without prejudice and replace with new claims 42-88 as follows:

Express Mail Label No. EJ915219874US

09/868677

--42. (New) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of a receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

43. (New) The isolated nucleic acid molecule of claim 42, wherein the receptor binding domains of the first and second subunits are the receptor binding domain from the same ligand.

44. (New) The isolated nucleic acid molecule of claim 42, wherein the receptor binding domain of the first subunit is a receptor binding domain derived from a different ligand than the receptor binding domain of the second subunit.

45. (New) The isolated nucleic acid molecule of claim 43, wherein the receptor binding domain of the first and second subunit is the fibrinogen domain of angiopoietin-1.

46. (New) The isolated nucleic acid molecule of claim 43, wherein the receptor binding domain of the first and second subunit is the fibrinogen domain of angiopoietin-2.

47. (New) The isolated nucleic acid molecule of claim 44, wherein the receptor binding domain of the first subunit is the fibrinogen domain of angiopoietin-1 and the receptor binding domain of the second subunit is the fibrinogen domain of angiopoietin-2.

48. (New) The isolated nucleic acid molecule of claim 44, wherein the receptor binding domain of the first subunit is the fibrinogen domain of angiopoietin-2 and the receptor binding domain of the second subunit is the fibrinogen domain of angiopoietin-1.

49. (New) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of the receptor binding domain of angiopoietin-1, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of angiopoietin-1.

50. (New) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of a receptor binding domain of angiopoietin-2, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of angiopoietin-2.

51. (New) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of a receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

52. (New) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of a receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

53. (New) The isolated nucleic acid molecule of claim 43, wherein the ligand is selected from the group consisting of the EPH family of ligands.

54. (New) The isolated nucleic acid molecule of claim 44, wherein the ligands are selected from the group consisting of the EPH family of ligands.
55. (New) The isolated nucleic acid molecule of claim 42, wherein the multimerizing component comprises an immunoglobulin derived domain.
56. (New) The isolated nucleic acid molecule of claim 43, wherein the multimerizing component comprises an immunoglobulin derived domain.
57. (New) The isolated nucleic acid molecule of claim 44, wherein the multimerizing component comprises an immunoglobulin derived domain.
58. (New) The isolated nucleic acid molecule of claim 55, 56, or 57, wherein the immunoglobulin derived domain is selected from the group consisting of the constant region domain of IgG, Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.
59. (New) A fusion polypeptide encoded by the isolated nucleic acid molecule of claims 42, 43, or 44.
60. (New) The fusion polypeptide of claim 59, wherein the fusion polypeptide is multimerized.
61. (New) A composition comprising the multimerized fusion polypeptide of claim 60.
62. (New) The composition of claim 61, wherein the multimer is a dimer.
63. (New) A vector which comprises the isolated nucleic acid molecule of claims 42, 43, or 44.
64. (New) An expression vector comprising a isolated nucleic acid molecule of claims 42, 43, or 44, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

65. (New) A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 64, in a suitable host cell.
66. (New) The host-vector system of claim 65, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.
67. (New) The host-vector system of claim 66, wherein the suitable host cell is E. coli.
68. (New) The host-vector system of claim 66, wherein the suitable host cell is a COS cell.
69. (New) The host-vector system of claim 66, wherein the suitable host cell is a CHO cell.
70. (New) A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 66, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.
71. (New) An isolated nucleic acid molecule encoding a fusion polypeptide, wherein the fusion polypeptide comprises more than one copy of a receptor binding domain of a ligand, each copy fused in tandem, and wherein either the N-terminal or the C-terminal ends of the tandem receptor binding domains is fused to a multimerizing component.
72. (New) The isolated nucleic acid molecule of claim 71, wherein the receptor binding domains are fused contiguously.
73. (New) The isolated nucleic acid molecule of claim 71 or 72, wherein the ligand is not a member of the EPH family of ligands.
74. (New) The isolated nucleic acid molecule of claim 71 or 72, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

75. (New) The isolated nucleic acid molecule of claim 71 or 72, wherein the multimerizing component comprises an immunoglobulin derived domain.

76. (New) The isolated nucleic acid molecule of claim 75, wherein the immunoglobulin derived domain is selected from the group consisting of the constant region domain of IgG, the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

77. (New) A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 71.

78. (New) The fusion polypeptide of claim 77, wherein the fusion polypeptide is multimerized.

79. (New) A composition comprising the multimerized fusion polypeptide of claim 78.

80. (New) The composition of claim 79, wherein the multimerized fusion polypeptide is a dimer.

81. (New) A vector which comprises the isolated nucleic acid molecule of claim 71.

82. (New) An expression vector comprising a nucleic acid molecule of claim 71, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

83. (New) A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 82, in a suitable host cell.

84. (New) The host-vector system of claim 83, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.

85. (New) The host-vector system of claim 84, wherein the suitable host cell is E. coli.

86. (New) The host-vector system of claim 84, wherein the suitable host cell is a COS cell.

87. (New) The host-vector system of claim 84, wherein the suitable host cell is a CHO cell.

88. (New) A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 83, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.--

Support for new claims 42-88 can be found throughout the specification.

In the specification

On page 1, line 3, before "This", please insert the following claim priority data:
This application is a U.S. National Stage Application of PCT International Application No. PCT/US99/30900, filed December 23, 1999, which claims priority to U.S. Provisional Application No. 60/113,387, filed December 23, 1998, now abandoned.

REMARKS

Attached herewith as Exhibit A are marked up copies of the amended pages and as Exhibit B are substitute sheets of the amended pages.

Applicants have canceled claims 1-41 and replaced them with new claims 42-88. Therefore, Applicants will consider new claims 42-88 for the purpose of calculating the filing fees associated with the U.S. National Stage Application filed concurrently herewith. No fee is deemed necessary in connection with filing this Preliminary Amendment. However, if any fee is deemed necessary,

REG 670A-US
Preliminary Amendment
Davis, et al.

authorization is hereby given to charge the amount of any such fee to Deposit
Account No. 18-0650.

Respectfully submitted,



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METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

This application is a U.S. National Stage application of PCT International application No. PCT/US 99/30900, filed December 23, 1999, which

This application claims priority of U.S. Application No. 60/113,387, filed December 23, 1998. Throughout this application, various publications are
5 cited. The disclosures of each and all of those publications are hereby incorporated by reference in their entireties into this application.

INTRODUCTION

10 The present invention provides for novel methods for producing novel fusion polypeptide ligands that have enhanced biological activity as compared to the polypeptide ligands in their native form. The invention also provides for nucleic acids useful for producing biologically active fusion polypeptide ligands, and the fusion polypeptide ligands themselves.

BACKGROUND OF THE INVENTION

The ability of polypeptide ligands to bind cells and thereby elicit a phenotypic response such as cell growth, survival or differentiation is often
20 mediated through transmembrane tyrosine kinases. The extracellular portion of each receptor tyrosine kinase (RTK) is generally the most distinctive portion of the molecule, as it provides the protein with its ligand-recognizing characteristic. Binding of a ligand to the extracellular domain results in signal transduction via an intracellular tyrosine kinase
25 catalytic domain which transmits a biological signal to intracellular target proteins. The particular array of sequence motifs of this cytoplasmic, catalytic domain determines its access to potential kinase substrates (Mohammadi, et al., 1990, Mol. Cell. Biol., 11: 5068-5078; Fantl, et al., 1992, Cell, 69:413-413).

30 RTKs appear to undergo dimerization or some related conformational change following ligand binding (Schlessinger, J., 1988, Trend Biochem. Sci.

SUBSTITUTE SHEET

METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

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METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

This application claims priority of U.S. Application No. 60/113,387, filed December 23, 1998. Throughout this application, various publications are cited. The disclosures of each and all of those publications are hereby incorporated by reference in their entireties into this application.

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13:443-447; Ullrich and Schlessinger, 1990, Cell, 61:203-212; Schlessinger and Ullrich, 1992, Neuron 9:383-391); molecular interactions between dimerizing cytoplasmic domains lead to activation of kinase function. In some instances, such as the growth factor platelet derived growth factor (PDGF), the ligand is a dimer that binds two receptor molecules (Hart, et al., 5 1988, Science, 240: 1529-1531; Heldin, 1989, J. Biol. Chem. 264:8905-8912) while, for example, in the case of EGF, the ligand is a monomer (Weber, et al., 1984, J. Biol. Chem., 259:14631-14636).

10 Throughout the history of the biotechnology industry, many novel genes and associated proteins have been identified by virtue of their sequence homology with known genes. Many such proteins are purported to be receptors, but since their cognate ligands have not been identified, they are referred to as orphan receptors. The screening of many of these orphan 15 receptors often leads to the identification of ligands that are capable of binding to the receptor, although the binding is often not associated with activation of any intracellular kinases or any other phenotypic change. Such was the case for members of the Eph receptor family. For sake of clarity, applicants incorporate by reference herein a letter cited as Eph 20 Nomenclature Committee, 1997, published in Cell vol. 90: 403-403 (1997) which sets forth a nomenclature for the Eph Receptor and Eph Ligand Families.

25 Little, if any, biological activity had been observed in response to binding of a ligand to an Eph family receptor prior to the discovery as set forth in U.S. Patent No. 5,747,033 issued May 5, 1998. That patent describes the concept of "clustering" whereby the soluble domains of ligands were combined to create multimers capable of activating the cognate receptors. Applicants have now extended the concept of clustering to additional ligands outside 30 the Eph family, for example, the Tie-2 receptor ligands known as the angiopoietins, and have also discovered that this method for production of homogeneous forms of clustered ligands is broadly applicable to improve

the affinity and/or increase the activity of a ligand as compared to the native form of the ligand.

Angiopoietin-1 (Ang) is one of two known ligands for the Tie-2 receptor and has been shown to be an agonist for Tie-2 (Davis, et al, 1996, Cell 87:1161-1169), whereas the second known ligand, angiopoietin-2, has been shown to be a naturally occurring antagonist of the Tie-2 receptor (Maisonpierre, et al., 1997, Science 277:55-60). Ang1* is a mutant form of angiopoietin-1 that comprises the N-terminal domain of angiopoietin-2 fused to the coiled-coil domain and the fibrinogen domain of angiopoietin-1 and that has a Cys to Ser mutation at amino acid 245. Ang1* has been shown to be a potent agonist for the Tie-2 receptor.

Experiments with mutants of angiopoietin-1 and angiopoietin-2 have demonstrated that the fibrinogen domains (FD) are the receptor-binding domains, and that dimerized versions of, for example Ang-1-FD-Fc, (i.e., the fibrinogen domain of Ang-1 fused to an Fc domain), can bind to the Tie-2 receptor with much higher affinity than monomeric Ang-1-FD (dimerization occurs due to the interaction between the Fc components of adjacent molecules). However, Ang-1-FD-Fc is not able to induce phosphorylation (activate) the Tie-2 receptor on endothelial cells unless it is further clustered with goat anti-human Fc antibodies (Jackson Immunoresearch). For this reason, mutant versions of Ang-1-FD and Ang-2-FD (i.e., the fibrinogen domain of Ang-2) were designed that were intrinsically more highly clustered.

SUMMARY OF THE INVENTION

The present invention provides for novel, biologically active, soluble forms of polypeptide ligands that bind to receptors on cells. Such polypeptide ligands are useful in promoting a differential function and/or influencing the phenotype, such as growth and/or proliferation, of receptor-bearing

cells. The invention also provides for nucleic acids encoding such polypeptide ligands, and both prokaryotic and eukaryotic expression systems for producing such polypeptide ligands. According to the invention, soluble forms of the polypeptide ligands described herein may be used to promote biological responses in receptor-expressing cells. In particular, a general method is described herein which produces fusion polypeptide ligands that may then be clustered, which functions to make otherwise inactive soluble polypeptide ligands biologically active, or which enhances the biological activity of polypeptide ligands that, absent such clustering, would have lower levels of biological activity. This method may be used to cluster a plurality of (more than one) receptor binding domains from any ligand which has improved affinity and/or increased activity (i.e. signaling ability) when clustered as compared to the native form of the ligand.

DESCRIPTION OF THE FIGURES

Figure 1A-1E - Nucleic acid sequence and deduced amino acid sequence of Ang-1-FD-FD-Fc.

Figure 2A-2E - Nucleic acid sequence and deduced amino acid sequence of Ang-2-FD-FD-Fc.

Figure 3A-3E - Nucleic acid sequence and deduced amino acid sequence of Ang-1-FD-Fc-FD.

Figure 4A-4E - Nucleic acid sequence and deduced amino acid sequence of Ang-2-FD-Fc-FD.

Figure 5 - Molecular Weight Analysis of Ang-1-FD-Fc-FD protein. SDS PAGE analyses showing a band running at about 210kD under non-reducing conditions (lane 3) and a band running at about 85kD under reducing conditions (lane 7).

Figure 6 - Light scatter analysis to confirm the molecular weight of Ang-1-FD-Fc-FD and to determine whether or not the protein is a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent is monitored with an on line light scattering detector and a refractive index and/or a UV detector. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) is used to calculate the protein concentration. The molecular weight of protein is then calculated from the angular dependence of light scattering. The molecular weight of the dimeric protein appears to be approximately 200kD and presence of a single peak implies that the protein solution is homogenous.

Figure 7 - Molecular Weight Analysis of Ang-2-FD-Fc-FD. SDS PAGE analyses showing a band running at about 200kD under non-reducing conditions (lanes 7 and 8) and a band running at about 88kD under reducing conditions (lanes 3 and 4).

Figure 8 - Light scatter analysis to confirm the molecular weight of Ang-2-FD-Fc-FD and to determine whether or not the protein is a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent is monitored with an on line light scattering detector and a refractive index and/or a UV detector. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) is used to calculate the protein concentration. The molecular weight of protein is then calculated from the angular dependence of light scattering. The molecular weight of the dimeric protein appears to be approximately 171kD and presence of a single peak implies that the

protein solution is homogenous.

Figure 9 - Ang1*-mediated vs. Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. A standard phosphorylation assay
5 revealed that Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926.

Figure 10 - Ability of Ang-2-FD-Fc-FD to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells. In a standard phosphorylation
10 assay, Ang-2-FD-Fc-FD is able to block Ang1* stimulation of the Tie-2 receptor when it is present in at least a 10-15 fold molar excess of Ang1*.

Figure 11 - Ability of angiopoietin-2 to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells. In a standard phosphorylation assay, at a
15 20 fold molar excess, angiopoietin-2 is not able to reduce the Ang1*-mediated phosphorylation level to 50%. This result, coupled with the results described in Figure 10 implies that Ang-2-FD-Fc-FD is a more potent inhibitor of Ang1*-mediated Tie-2 receptor phosphorylation than angiopoietin-2.

Figure 12 - Ability of Ang-2-FD-Fc-FD to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells. In a standard phosphorylation assay, it is shown that while there is a trend toward
20 blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, Ang-2-FD-Fc-FD seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 10.

Figure 13 - Ability of angiopoietin-2 to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells. In a standard
30 phosphorylation assay, it is shown that there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, but, like Ang-2-FD-Fc-FD, angiopoietin-2 seems to be more effective at

blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 11.

Figure 14A-14E - Nucleic acid sequence and deduced amino acid sequence of Ephrin-B1-Ephrin-B1-Fc.

5

Figure 15A-15E - Nucleic acid sequence and deduced amino acid sequence of Ephrin-B2-Ephrin-B2-Fc.

Figure 16 - Comparison of Ephrin-B1-Fc, Ephrin-B1-Ephrin-B1-Fc, Ephrin-

10 B2-Fc and Ephrin-B2-Ephrin-B2-Fc in standard EphB2 phosphorylation assays. COS cells were serum-starved and then left untreated (UT), lane 1, or were treated with unclustered and clustered Ephrin-B1-Fc (Efn-B1), lanes 2 and 3. COS cells were also treated with unclustered and clustered Ephrin-B1-Ephrin-B1-Fc (Efn-B1 DD), lanes 4 and 5. In addition cells were likewise
15 treated with unclustered and clustered Ephrin-B2-Fc (Efn-B2), lanes 6 and 7 and with unclustered and clustered Ephrin-B2-Ephrin-B2-Fc (Efn-B2 DD), lanes 8 and 9. The extent of EphB2 phosphorylation was assessed by anti-phosphotyrosine western blotting (upper panels) and the relative amounts of EphB2 in each lane was determined by anti-EphB2 western blotting
20 (lower panels).

Figure 17 - Ang1*-mediated vs. stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. EAhy926 cells were stimulated with 0.4 µg/ml Ang1* or 0.2 µg/ml or 0.4 µg/ml stable
25 CHO clone-derived Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that stable CHO clone-derived Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells.

30 Figure 18 - Ability of stable CHO clone-derived Ang-2-FD-Fc-FD to block stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. EAhy926 cells were treated with 0.2

µg/ml of the Tie-2 agonist Ang-1-FD-Fc-FD and 2 µg/ml, 4 µg/ml, 8 µg/ml or 16 µg/ml of stable CHO clone-derived Ang-2-FD-Fc-FD. Ang-2-FD-Fc-FD is able to block or stable CHO clone-derived Ang-1-FD-Fc-FD stimulation of the Tie-2 receptor when it is present in at least a 40 fold molar excess of stable CHO clone-derived Ang-1-FD-Fc-FD.

DETAILED DESCRIPTION OF THE INVENTION

As described in greater detail below, applicants have discovered a method for "clustering" polypeptide ligands, which functions to make otherwise inactive soluble polypeptide ligands biologically active, or which enhances the biological activity of polypeptide ligands that, absent such clustering, would have lower levels of biological activity. This method may be used to cluster a plurality of (more than one) receptor binding domains from any ligand which has improved affinity and/or increased activity (i.e. signaling ability) when clustered as compared to the native form of the ligand.

The present invention provides for a nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of the receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, said multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

In one embodiment of the invention, the receptor binding domains of the first and second subunits are copies of the receptor binding domain of the same ligand. The first and second subunits may each have one or more than one copy of the receptor binding domain of the ligand. In specific embodiments of the invention, the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2. Alternatively, the

receptor binding domain is from a ligand selected from the group consisting of the EPH family of ligands (i.e., the ephrins).

In another embodiment of the invention, the receptor binding domains of the first subunit are copies of the receptor binding domain of a different ligand from the receptor binding domains of the second subunit. For example, the first subunit may comprise the receptor binding domain of an angiopoietin and the second subunit may comprise the receptor binding domain of vascular endothelial growth factor (VEGF). Alternatively, the first subunit may comprise the receptor binding domain of VEGF and the second subunit may comprise the receptor binding domain an angiopoietin. Still further, the first and second subunits may each have one or more than one copy of the receptor binding domain of their respective ligand.

By "receptor binding domain" what is meant is the minimal portion of the ligand that is necessary to bind its receptor.

In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as Fc(Δ C1).

The present invention also provides for fusion polypeptides encoded by the nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are described in European Patent Application of ZymoGenetics, Inc., Publication No. EP 0 721 983 A1 published 17 July 1996

and include S. cerevisiae repressible acid phosphatase (Mizunaga et al., 1988, J. Biochem. (Tokyo) 103:321-326); the S. cerevisiae type 1 killer preprotoxin (Sturley et al., 1986, EMBO J. 5:3381-3390); the S. calbergensis alpha galactosidase melibiase (Sumner-Smith, et al., 1985, Gene 36:333-340); and
5 the Neurospora crassa ornithine decarboxylase (Digangi, et al., 1987, J. Biol. Chem. 262:7889-7893). Sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell 29:671-679); the S. cerevisiae SUC2 gene (Carlson et al., 1983, Mol. Cell. Biol. 3:439-447); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the
10 invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which
20 comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion polypeptide. The suitable host cell may be a bacterial cell such as E. coli, a yeast cell, such as Pichia pastoris, an insect cell, such as Spodoptera frugiperda, or a mammalian cell, such as a COS or CHO cell.

25 The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

30 The fusion polypeptides useful for practicing the present invention may be prepared by expression in a prokaryotic or eukaryotic expression system.

The recombinant gene may be expressed and the polypeptide purified utilizing any number of methods. The gene may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

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The fusion polypeptides may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

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The present invention also provides for a nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises more than one copy of the receptor binding domain of a ligand in tandem, and wherein either the N-terminal or the C-terminal receptor binding domain is also fused to a multimerizing component. In one embodiment of the invention, the receptor binding domains are fused contiguously. In another embodiment of the invention, the receptor binding domains are from a ligand that is not a member of the EPH family of ligands (i.e., not an ephrin). In specific embodiments of the invention, the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2. In an alternative embodiment, the receptor binding domain is from vascular endothelial growth factor (VEGF). In another embodiment, the receptor binding domain is from an ephrin.

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By "receptor binding domain" what is meant is the minimal portion of the ligand that is necessary to bind its receptor.

In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as Fc(Δ C1).

The present invention also provides for fusion polypeptides encoded by the nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are described in European Patent Application of ZymoGenetics, Inc., Publication No. EP 0 721 983 A1 published 17 July 1996 and include *S. cerevisiae* repressible acid phosphatase (Mizunaga et al., 1988, J. Biochem. (Tokyo) 103:321-326); the *S. cerevisiae* type 1 killer preprotoxin (Sturley et al., 1986, EMBO J. 5:3381-3390); the *S. caltsbergensis* alpha galactosidase melibiase (Sumner-Smith, et al., 1985, Gene 36:333-340); and the *Neurospora crassa* ornithine decarboxylase (Digangi, et al., 1987, J. Biol. Chem. 262:7889-7893). Sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell 29:671-679); the *S. cerevisiae* *SUC2* gene (Carlson et al., 1983, Mol. Cell. Biol. 3:439-447); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is

operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion polypeptide.

5 The suitable host cell may be a bacterial cell such as E. coli, a yeast cell, such as Pichia pastoris, an insect cell, such as Spodoptera frugiperda, or a mammalian cell, such as a COS or CHO cell.

10 The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

15 The fusion polypeptides useful for practicing the present invention may be prepared by expression in a prokaryotic or eukaryotic expression system. The recombinant gene may be expressed and the polypeptide purified utilizing any number of methods. The gene may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

20 The fusion polypeptides may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may
25 be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

30 The Examples describe the preparation of novel polypeptide ligands that comprise a receptor binding domain of a member of the Eph (Eph transmembrane tyrosine kinase family ligands) family of ligands or of a

member of the angiopoietin family of ligands that can bind the Tie-2 receptor.

For a description of novel Eph family ligands, methods of making and using them, as well as the sequences of EHK-1L, B61 and ELK-L, together with a description of a method of enhancing the biological activity of EPH family ligands by clustering them, applicants refer to U.S. Patent No. 5,747,033 issued on May 5, 1998 which is hereby incorporated by reference in its entirety. Applicants further refer to International Application PCT/US93/10879, published as WO 94/11020 on May 26, 1994; and International Application PCT/US96/17201 published as WO 97/15667 entitled "Biologically Active EPH Family Ligands" each of which is hereby incorporated by reference in its entirety.

As has been previously reported, a family of ligands for the TIE-2 receptor has been discovered and named the Angiopoietins. This family, consisting of TIE-2 ligand 1 (Ang-1); TIE-2 ligand 2 (Ang-2); TIE ligand 3 (Ang-3); and TIE ligand 4 (Ang-4) has been extensively characterized. For a description of the cloning, sequencing and characterization of the angiopoietins, as well as for methods of making and uses thereof, including the production and characterization of modified and chimeric ligands thereof, reference is hereby made to the following publications, each of which is incorporated by reference herein in its entirety: U.S. Patent No. 5,521,073 issued May 28, 1996; U.S. Patent No. 5,643,755 issued July 1, 1997; U.S. Patent No. 5,650,490 issued July 22, 1997; U.S. Patent No. 5,814,464 issued September 29, 1998; U.S. Patent No. 5,879,672 issued March 9, 1999; U.S. Patent No. 5,851,797 issued December 22, 1998; PCT International Application entitled "TIE-2 Ligands Methods of Making and Uses Thereof," published as WO 96/11269 on 18 April 1996 in the name of Regeneron Pharmaceuticals, Inc.; PCT International Application entitled "TIE-2 Ligands Methods of Making and Uses Thereof," published as WO 96/31598 on 10 October 1996 in the name of Regeneron Pharmaceuticals, Inc.; PCT International Application entitled

“TIE-2 Receptor Ligands (TIE Ligand-3; TIE Ligand-4) And Their Uses,”
published as WO 97/48804 on 24 December 1997 in the name of Regeneron
Pharmaceuticals, Inc; and PCT International Application entitled “Modified
TIE-2 Receptor Ligands,” published as WO 98/05779 on 12 February 1998 in
5 the name of Regeneron Pharmaceuticals, Inc.

When used herein, fusion polypeptide includes functionally equivalent
molecules in which amino acid residues are substituted for residues within
the sequence resulting in a silent or conservative change. For example, one
10 or more amino acid residues within the sequence can be substituted by
another amino acid of a similar polarity which acts as a functional
equivalent, resulting in a silent or conservative alteration. Substitutes for
an amino acid within the sequence may be selected from other members of
the class to which the amino acid belongs. For example, the nonpolar
15 (hydrophobic) amino acids include alanine, leucine, isoleucine, valine,
proline, phenylalanine, tryptophan and methionine. The polar neutral
amino acids include glycine, serine, threonine, cysteine, tyrosine,
asparagine, and glutamine. The positively charged (basic) amino acids
include arginine, lysine and histidine. The negatively charged (acidic)
20 amino acids include aspartic acid and glutamic acid. Also included within
the scope of the invention are proteins or fragments or derivatives thereof
which exhibit the same or similar biological activity and derivatives which
are differentially modified during or after translation, *e.g.*, by glycosylation,
proteolytic cleavage, linkage to an antibody molecule or other cellular
25 ligand, etc.

Cells that express the fusion polypeptides of the invention are genetically
engineered to produce them by, for example, transfection, transduction,
electroporation, or microinjection.

The present invention encompasses the nucleic acid sequences encoding the fusion polypeptides of the invention, as well as sequences that hybridize under stringent conditions to nucleic acid sequences that are

5 complementary to the nucleic acid sequences of the invention. Stringent conditions are set forth in, for example, Sambrook, et al. Molecular Cloning: A Laboratory Manual, 2 ed. Vol. 1, pp. 101-104, Cold Spring Harbor Laboratory Press (1989). In addition, the present invention encompasses nucleic acid sequences that are different from the nucleic acid sequences of
10 the invention but which nevertheless encode the fusion polypeptides of the invention due to the degeneracy of the genetic code.

In addition, the present invention contemplates use of the fusion polypeptides described herein in tagged forms.

15 Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors encoding the fusion polypeptides of the invention using appropriate transcriptional/translational control signals and the protein coding
20 sequences. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinations (genetic recombination). Expression of nucleic acid sequence encoding the fusion polypeptides of the invention may be regulated by a second nucleic acid sequence so that the fusion polypeptide is expressed in a host transformed with the recombinant
25 DNA molecule. For example, expression of the fusion polypeptides described herein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the fusion polypeptide include, but are not limited to the long terminal repeat as described in Squinto et al., (1991, Cell 65:1-20); the SV40 early
30 promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980,

Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:144-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the b-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25), see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58); alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al, 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogam et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene

control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Thus, according to the invention, expression vectors capable of being
5 replicated in a bacterial or eukaryotic host comprising Eph fusion
polypeptide encoding or angiopoietin fusion polypeptide encoding nucleic
acids as described herein, are used to transfect the host and thereby direct
expression of such nucleic acid to produce fusion polypeptides which may
then be recovered in biologically active form. As used herein, a biologically
10 active form includes a form capable of binding to the relevant receptor and
causing a differentiated function and/or influencing the phenotype of the
cell expressing the receptor. Such biologically active forms would, for
example, induce phosphorylation of the tyrosine kinase domain of the Etk-
1, Elk, or Tie2 receptor, or stimulation of synthesis of cellular DNA.

15 Expression vectors containing the nucleic acid inserts can be identified by
three general approaches: (a) DNA-DNA hybridization, (b) presence or
absence of "marker" gene functions, and (c) expression of inserted
sequences. In the first approach, the presence of a foreign nucleic acids
20 inserted in an expression vector can be detected by DNA-DNA hybridization
using probes comprising sequences that are homologous to an inserted
nucleic acid sequences. In the second approach, the recombinant
vector/host system can be identified and selected based upon the presence
or absence of certain "marker" gene functions (*e.g.*, thymidine kinase
25 activity, resistance to antibiotics, transformation phenotype, occlusion body
formation in baculovirus, etc.) caused by the insertion of foreign nucleic
acid sequences in the vector. For example, if an *efl* nucleic acid sequence is
inserted within the marker gene sequence of the vector, recombinants
containing the insert can be identified by the absence of the marker gene
30 function. In the third approach, recombinant expression vectors can be
identified by assaying the foreign nucleic acid product expressed by the
recombinant. Such assays can be based, for example, on the physical or

functional properties of the nucleic acid product of interest, for example, by binding of a ligand to a receptor or portion thereof which may be tagged with, for example, a detectable antibody or portion thereof or binding to antibodies produced against the protein of interest or a portion thereof.

5

Cells of the present invention may transiently or, preferably, constitutively and permanently express the ephrin or angiopoietin fusion polypeptide as described herein.

10 The ephrin fusion polypeptides of the invention may be useful in methods of treating a patient suffering from a neurological disorder comprising treating the patient with an effective amount of the ephrin fusion polypeptide.

15 For example, the Elk receptor is expressed primarily in brain. Accordingly, it is believed that an Elk binding ephrin fusion polypeptide ligand will support the induction of a differential function and/or influence the phenotype, such as growth and/or survival of neural cells that express this receptor.

20

The present invention also provides for pharmaceutical compositions comprising the ephrin fusion polypeptide in a suitable pharmacologic carrier. The compositions may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, including, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

25

As our understanding of neurodegenerative disease/neurotrauma becomes clearer, it may become apparent that it would be beneficial to decrease the effect of endogenous Efl-6. Therefore, in areas of nervous system trauma, it may be desirable to provide Efl-6 antagonists, including, but not limited to,

30

fusion polypeptide forms of Efl-6 which may compete with cell-bound ligand for interaction with Elk receptor. It may be desirable to provide such antagonists locally at the injury site rather than systemically. Use of an Efl-6 antagonist providing implant may be desirable.

5

Alternatively, certain conditions may benefit from an increase in Efl-6 responsiveness. It may therefore be beneficial to increase the number or binding affinity of Efl-6 in patients suffering from such conditions.

10

The invention herein further provides for the development of a fusion polypeptide, as a therapeutic for the treatment of patients suffering from disorders involving cells, tissues or organs which express the TIE-2 receptor. Such molecules may be used in a method of treatment of the human or animal body, or in a method of diagnosis.

15

Because TIE-2 receptor has been identified in association with endothelial cells and, as was previously demonstrated, blocking of agonists of the receptor such as TIE-2 ligand 1 (Ang-1) has been shown to prevent vascularization, applicants expect that TIE-2 agonist fusion polypeptides of the invention may be useful for the induction of vascularization in diseases or disorders where such vascularization is indicated. Such diseases or disorders would include wound healing, ischemia and diabetes. The ligands may be tested in animal models and used therapeutically as described for other agents, such as vascular endothelial growth factor

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(VEGF), another endothelial cell-specific factor that is angiogenic. Ferrara, et al. U.S. Patent No. 5,332,671 issued July 26, 1994. The Ferrara reference, as well as other studies, describe in vitro and in vivo studies that may be used to demonstrate the effect of an angiogenic factor in enhancing blood flow to ischemic myocardium, enhancing wound healing, and in other therapeutic settings wherein neoangiogenesis is desired. [see Sudo, et al., European Patent Application 0 550 296 A2 published July 7, 1993; Banai, et al. Circulation 89:2183-2189 (1994); Unger, et al. Am. J. Physiol. 266:H1588-H1595

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(1994); Lazarous, et al. Circulation 91:145-153 (1995)]. According to the invention, the agonist fusion polypeptides may be used alone or in combination with one or more additional pharmaceutically active compounds such as, for example, VEGF or basic fibroblast growth factor (bFGF).

Conversely, antagonists of the TIE-2 receptor, such as TIE-2 receptorbodies or TIE-2 ligand 2 (Ang-2) as described in Example 9 in International Publication No. WO 96/31598 published 10 October 1996, have been shown to prevent or attenuate vascularization, and are thus expected to be useful in preventing or attenuating, for example, tumor growth. Similarly then, TIE-2 antagonist fusion polypeptides of the invention would also be useful for those purposes. These antagonists may be used alone or in combination with other compositions, such as anti-VEGF antibodies, that have been shown to be useful in treating conditions in which the therapeutic intent is to block angiogenesis.

For example, applicants have determined that TIE-2 ligands are expressed in cells within, or closely associated with, tumors. For example, TIE-2 ligand 2 (Ang-2) appears to be tightly associated with tumor endothelial cells. Accordingly, TIE-2 antagonist fusion polypeptides of the invention may also be useful in preventing or attenuating, for example, tumor growth.

In other embodiments, the TIE-2 agonist fusion polypeptides of the invention described herein may be used as hematopoietic factors. A variety of hematopoietic factors and their receptors are involved in the proliferation and/or differentiation and/or migration of the various cells types contained within blood. Because the TIE-2 receptors are expressed in early hematopoietic cells, the TIE-2 ligands are expected to play a comparable role in the proliferation or differentiation or migration of these cells. Thus, for example, TIE-2 agonist fusion polypeptide compositions may be prepared, assayed, examined in in vitro and in vivo biological systems and

used therapeutically as described in any of the following: Sousa, U.S. Patent No. 4,810,643, Lee, et al., Proc. Natl. Acad. Sci. USA 82:4360-4364 (1985)

Wong, et al. Science, 228:810-814 (1985); Yokota, et al. Proc. Natl. Acad. Sci

(USA) 81:1070 (1984); Bosselman, et al. WO 9105795 published May 2, 1991

5 entitled "Stem Cell Factor" and Kirkness, et al. WO 95/19985 published July 27, 1995 entitled "Haemopoietic Maturation Factor". Accordingly, the

fusion polypeptides may be used to diagnose or treat conditions in which normal hematopoiesis is suppressed, including, but not limited to anemia, thrombocytopenia, leukopenia and granulocytopenia. In a preferred

10 embodiment, the fusion polypeptides may be used to stimulate

differentiation of blood cell precursors in situations where a patient has a disease, such as acquired immune deficiency syndrome (AIDS) which has

caused a reduction in normal blood cell levels, or in clinical settings in

which enhancement of hematopoietic populations is desired, such as in

15 conjunction with bone marrow transplant, or in the treatment of aplasia or myelosuppression caused by radiation, chemical treatment or chemotherapy.

The fusion polypeptides of the present invention may be used alone, or in

20 combination with another pharmaceutically active agents such as, for example, cytokines, neurotrophins, interleukins, etc. In a preferred

embodiment, the fusion polypeptides may be used in conjunction with any of a number of factors which are known to induce stem cell or other

hematopoietic precursor proliferation, or factors acting on later cells in the

25 hematopoietic pathway, including, but not limited to, hemopoietic

maturation factor, thrombopoietin, stem cell factor, erythropoietin, G-CSF, GM-CSF, etc.

In an alternative embodiment, TIE-2 receptor antagonist fusion

30 polypeptides are used to diagnose or treat patients in which the desired

result is inhibition of a hematopoietic pathway, such as for the treatment of myeloproliferative or other proliferative disorders of blood forming organs

such as thrombocythemias, polycythemias and leukemias. In such embodiments, treatment may comprise use of a therapeutically effective amount of the fusion polypeptides as described herein.

5 Effective doses useful for treating these or other diseases or disorders may be determined using methods known to one skilled in the art [see, for example, Fingl, et al., The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds. Macmillan Publishing Co., New York, pp. 1-46 ((1975))]. Pharmaceutical compositions for use according to the invention include the
10 fusion polypeptides described above in a pharmacologically acceptable liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation prior to administration *in vivo*. For example, the pharmaceutical composition may
15 comprise a fusion polypeptide in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such treatment. The administration route may be any mode of administration
20 known in the art, including but not limited to intravenously, intrathecally, subcutaneously, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

Administration may result in the distribution of the active agent of the
25 invention throughout the body or in a localized area. For example, in some conditions which involve distant regions of the nervous system, intravenous or intrathecal administration of agent may be desirable. In some situations, an implant containing active agent may be placed in or near the lesioned area. Suitable implants include, but are not limited to,
30 gelfoam, wax, or microparticle-based implants.

The present invention also provides for pharmaceutical compositions comprising the fusion polypeptides described herein, in a pharmacologically acceptable vehicle. The compositions may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, including, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLES

Angiopoietin ligands:

As described *supra*, experiments with mutants of Ang-1 and Ang-2 have demonstrated that the fibrinogen domains (FD) are the receptor-binding domains, and that dimerized versions (dimerization occurs due to the interaction between the Fc components of adjacent molecules), for example Ang-1-FD-Fc, can bind to the Tie-2 receptor with much higher affinity than monomeric Ang-1-FD. However, Ang-1-FD-Fc is not able to induce phosphorylation (activate) the Tie-2 receptor on endothelial cells unless it is further clustered with goat anti-human Fc antibodies (Jackson ImmunoResearch). For this reason, mutant versions of Ang-1-FD and Ang-2-FD were designed that were intrinsically more highly clustered.

Two general types of nucleic acid molecules were constructed. The first type consisted of two tandem copies of Ang-1-FD fused to an Fc tag, thus leading

to a secreted polypeptide molecule that is dimeric with respect to the Fc tag but tetrameric with respect to Ang-1-FD. Similarly, two tandem copies of Ang-2-FD fused to an Fc tag constituted the angiopoietin-2 version of this type of construct. These molecules were designated Ang-1-FD-FD-Fc and
5 Ang-2-FD-FD-Fc, respectively.

In the second type of nucleic acid molecule constructed, two copies of Ang-1-FD were connected by an Fc tag bridging between them, thus creating the structure Ang-1-FD-Fc-FD that is still dimeric with respect to the Fc, as well
10 as tetrameric with respect to Ang-1-FD. An angiopoietin-2 version was also constructed and these two molecules were designated Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD, respectively.

For either type of construct, similar properties were observed: unlike
15 dimeric Ang-1-FD-Fc, which fails to activate Tie-2 in endothelial cells, both Ang-1-FD-FD-Fc and Ang-1-FD-Fc-FD could readily activate Tie-2 in endothelial cells, with a potency comparable to that of the native ligand. Also, like native angiopoietin-2, Ang-2-FD-Fc-FD could antagonize angiopoietin-1 activity with a potency that is comparable to that of native
20 angiopoietin-2, and with much greater potency than the marginally antagonistic activity of the Ang-2-FD-Fc dimer.

Construction of mutant angiopoietin nucleic acid molecules.

25 All of the following nucleic acid molecules were constructed by standard recombinant DNA techniques (See e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY), sequence-verified by standard
30 techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA), and subcloned into the mammalian expression vector pMT21 (Genetics

Institute, Inc.) with a Kozak sequence (Kozak, M., 1987, Nucleic Acids Res. 15:8125-8148) at the 5' end to promote protein translation. The bridging sequences described *infra* were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains, but there is no indication that there is a very critical nature to these bridging sequences (though varying the length of the linker in some of these constructs led to some variation in the amount of protein produced).

Example 1: Construction of the Ang-1-FD-FD-Fc, Ang-2-FD-FD-Fc, Ang-1-FD-Fc-FD, and Ang-2-FD-Fc-FD nucleic acid molecules.

Ang-1-FD-FD-Fc: Ang-1-FD-FD-Fc consists of a trypsin signal sequence at its amino terminus to allow for secretion (bases 1-45 of Figure 1A) followed by the angiopoietin-1 fibrinogen domain (FD) (bases 46-690 of Figure 1A-Figure 1B), a short bridging sequence consisting of the amino acids Gly-Pro Ala-Pro (bases 691-702 of Figure 1B), a second angiopoietin-1 FD (bases 703-1750 of Figure 1B-Figure 1D), another bridging sequence consisting of the amino acids Gly-Pro-Gly (bases 1351-1359 of Figure 1D), and the coding sequence for the Fc portion of human IgG1 (bases 1360-2058 of Figure 1D-Figure 1E).

Ang-2-FD-FD-Fc: The Ang-2-FD-FD-Fc nucleic acid molecule was similarly constructed. It consists of a trypsin signal sequence (bases 1-45 of Figure 2A), an angiopoietin-2 FD (bases 46-690 of Figure 2A- Figure 2B), a bridging amino acid sequence Gly-Gly-Pro-Ala-Pro (bases 691-705 of Figure 2B), a second angiopoietin-2 FD (bases 706-1353 of Figure 2B-Figure 2D), another bridging amino acid sequence Gly-Pro-Gly (bases 1354-1362 of Figure 2D), and the coding sequence for the Fc portion of human IgG1 (bases 1363-2061 of Figure 2D-Figure 2E).

Ang-1-FD-Fc-FD: The Ang-1-FD-Fc-FD consists of a trypsin signal sequence (bases 1-45 of Figure 3A), an angiopoietin-1 FD (bases 46-690 of Figure 3A-3B), the bridging amino acid sequence Gly-Pro-Gly (bases 691-699 of Figure

3B), the coding sequence for the Fc portion of human IgG1 (bases 700-1395 of Figure 3B-3D), another bridging amino acid sequence Gly-Gly-Gly-Gly-Ser-Gly-Ala-Pro (bases 1396-1419 of Figure 3D), and a second angiopoietin-1 FD (bases 1420-2067 of Figure 3D-Figure 3E).

5 **Ang-2-FD-Fc-FD:** The Ang-2-FD-Fc-FD nucleic acid molecule consists of a trypsin signal sequence (bases 1-45 of Figure 4A), an angiopoietin-2 FD domain (bases 46-690 of Figure 4A-Figure 4B), the bridging amino acid sequence Gly-Gly-Pro-Gly (bases 691-702 of Figure 4B), the coding sequence
10 for the Fc portion of human IgG1 (bases 703-1398 of Figure 4B- Figure 4D), the bridging amino acid sequence Gly-Gly-Gly-Gly-Ser-Gly-Ala-Pro (bases 1399-1422 of Figure 4D), and a second angiopoietin-2 FD (bases 1423-2067 of Figure 4D-Figure 4E).

15 **Example 2: Characterization of Ang-1 FD-Fc-FD protein.**

Molecular Weight Analysis: The predicted molecular weight for Ang-1-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form (with
20 respect to the Fc) has a predicted weight of 76,349. In addition, there are three predicted N-linked glycosylation sites, approximately 2500 MW/site, that could potentially increase the molecular weight of the monomeric protein to 83,849. Due to the interaction between the Fc components of adjacent molecules, the protein actually exists as a dimer with a predicted
25 molecular weight, including possible N-linked glycosylation, of 167,698. Subsequent SDS PAGE analyses of COS cell-derived protein described *infra* confirmed these approximate molecular weights, with a band running at about 210kD under non-reducing conditions and a band running at about 85kD under reducing conditions (Figure 5). Light scatter analysis was
30 performed to further confirm the molecular weight and, more importantly, determine whether or not the protein was a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To

determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent was monitored with an on line light scattering detector and a refractive index and/or a UV detector. The light scattering detector is a MiniDawn laser light scattering detector was from
5 Wyatt Technology Corporation (Santa Barbara, CA). This instrument measures static light at three different angles. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) was used to calculate the protein concentration based on either dn/dc (dn = change of
10 refractive index; dc = concentration) or the extinction coefficient of the protein. The molecular weight of protein is then calculated from the angular dependence of light scattering. Figure 6 shows the results of this analysis using COS cell-derived protein. The molecular weight of the dimeric protein appears to be approximately 200kD and presence of a single
15 peak implies that the protein solution is, in fact, homogenous.

Expression Level in COS Cells: COS cell supernatant containing recombinant Ang-1-FD-Fc-FD protein was generated by transiently transfecting COS cells with the Ang1-FD-Fc-FD DNA construct described
20 *supra*. All transfections were performed using standard techniques known in the art. The COS cell supernatant was analyzed using Biacore technology (Pharmacia, Inc.) to quantitate the amount of Ang-1-FD-Fc-FD protein present in the supernatant. This analysis resulted in an RU value of 765, which is equivalent to 0.9mg of recombinant protein/liter of COS cell
25 supernatant. These values represent very high levels of expression.

Purification of COS Supernatants: Because the Ang-1-FD-Fc-FD protein contains an Fc domain, purification is relatively simple and straight forward using standard Protein A column chromatography (Pharmacia,
30 Inc.) followed by standard size exclusion chromatography (Pharmacia, Inc.). In fact, the relative ease of purification of the Ang-1-FD-Fc-FD protein gives it a distinct advantage over the parent protein, angiopoietin-1, from which

it is derived, and the mutant version of angiopoietin-1 called Ang1* that consists of the N-terminal of angiopoietin-2 fused to the coiled-coil domain and fibrinogen domain of angiopoietin-1 and that has a Cys to Ser mutation at amino acid 245. (See PCT International Application entitled "Modified
5 TIE-2 Receptor Ligands," published as WO 98/05779 on 12 February 1998 in the name of Regeneron Pharmaceuticals, Inc., especially Figure 27, which is hereby incorporated by reference).

Both angiopoietin-1 and Ang1* require extensive, expensive and labor-
10 intensive purification schemes that result in relatively poor yields of recombinant protein. The need for cost-effective, simple purification schemes for biologicals intended for clinical use can not be over-emphasized.

15 The COS cell supernatant was purified as described *supra* and yielded approximately 1 mg of purified Ang-1-FD-Fc-FD protein that was used in the studies described *infra* to further characterize the protein.

N-terminal sequencing of COS cell-derived Ang-1-FD-Fc-FD protein:

20 Purified Ang-1-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. This was of concern because the mutant molecule, Ang1*, has a history of containing between 10-20% N-terminally truncated species. The analysis revealed only one N-terminal sequence, Arg-Asp-X-Ala-Asp,
25 wherein X is Cys. This sequence can be found at amino acids 16-20 of Figure 3A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 Figure 3A.

Receptor binding analysis of COS cell-derived Ang-1-FD-Fc-FD: Previous

30 studies have determined that the fibrinogen domain (FD) of the angiopoietin molecules is necessary for ligand/receptor interaction. Furthermore, in order for high affinity binding to the Tie-2 receptor to

occur, native angiopoietin-1, angiopoietin-2, and the mutant Ang1* must form at least tetrameric, and possibly higher order, multimers. To determine whether the COS cell-derived Ang-1-FD-Fc-FD protein, which is tetrameric with respect to the FD domain, could bind to Tie-2 with high affinity, standard Biacore analysis was performed. Briefly, Tie-2-Fc receptor body protein, which is a fusion protein comprising the ectodomain of Tie-2 fused to the Fc domain of human IgG1, was immobilized on a Biacore chip. Ang-1-FD-Fc-FD-containing solution was passed over the chip and binding between Tie-2 ectodomain and Ang-1-FD-Fc-FD was allowed to occur. The binding step was followed by a 0.5 M NaCl high salt wash. The high salt wash was not able to disrupt the interaction between the Ang-1-FD-Fc-FD protein and the Tie-2 receptor ectodomain, implying that there is a strong interaction between the mutant ligand and receptor. This result is consistent with earlier Biacore results in which both Ang-1-FD-Fc-FD parent molecule, angiopoietin-1 and the mutant Ang1* molecule, have been shown to interact strongly with the Tie-2-Fc receptor and that this interaction is not disrupted by high salt. In contrast, several mutant molecules derived from the parent angiopoietin-1 molecule are readily dissociated from the Tie-2-Fc receptor when treated with high salt. The mutant molecules, designated Ang-1/FD (a monomer with respect to the FD), Ang-1/FD-Fc (also a monomer with respect to the FD, but which is able to form a dimer due to the presence of the Fc domain), and Ang-1/C/FD (a monomer with respect to the FD, but which also contains the coiled-coil domain of angiopoietin-1), do not exist in multimeric forms sufficient for high affinity binding to the Tie-2 receptor.

Example 3: Characterization of COS cell-derived Ang-2-FD-Fc-FD protein.

Molecular Weight Analysis: As described for Ang-1-FD-Fc-FD *supra*, the predicted molecular weight for Ang-2-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form of Ang-2-FD-Fc-FD has a predicted

weight of 76,052, with three predicted N-linked glycosylation sites that could potentially increase the molecular weight of the monomeric protein to 83,552. Like Ang-1-FD-Fc-FD, the protein exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,104.

5 SDS PAGE analyses of COS cell-derived protein confirmed these approximate molecular weights, with a band running at about 200kD under non-reducing conditions and a band running at about 88kD under reducing conditions (Figure 7). Light scatter analysis confirmed the molecular weight (171kD) and revealed that the Ang-2-FD-Fc-FD protein, like Ang-1-FD-Fc-FD, exists as a homogeneous species (Figure 8).

Expression Level in COS Cells: COS cell supernatant containing recombinant Ang-2-FD-Fc-FD protein was generated by transiently transfecting COS cells with the Ang-2-FD-Fc-FD DNA construct described *supra*. The COS cell supernatant was analyzed by Biacore to quantitate the amount of Ang-2-FD-Fc-FD protein present in the supernatant. This analysis resulted in an RU value of 606, which is equivalent to 0.7mg of recombinant protein/liter of COS cell supernatant. These values represent relatively high levels of expression.

Purification of COS Supernatants: As with Ang-1-FD-Fc-FD, Ang-2-FD-Fc-FD protein contains an Fc domain, so purification is relatively simple and straight forward using standard Protein A column chromatography followed by standard size exclusion chromatography. The COS cell supernatant was purified as described for Ang-1-FD-Fc-FD *supra* and yielded approximately 2 mg of purified Ang-2-FD-Fc-FD protein that was used in the studies described *infra* to further characterize this protein.

N-terminal sequencing: Purified COS cell-derived Ang-2-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal sequence, Arg-Asp-X-Ala-Glu, wherein X is Cys. This

sequence can be found at amino acids 16-20 of Figure 4A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 of Figure 4A.

5 **Receptor binding analysis of COS cell-derived protein:** To determine whether the COS cell-derived Ang-2-FD-Fc-FD protein could bind to the Tie-2 receptor, standard Biacore analysis was performed as described for Ang-1-FD-Fc-FD *supra*. As with Ang-1-FD-Fc-FD, a high salt wash was not able to disrupt the interaction between the Ang-2-FD-Fc-FD protein and the
10 Tie-2-Fc receptor, again implying that there is a strong interaction between mutant ligand and receptor.

Example 4: Effects of COS cell-derived Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD on Tie-2 receptor phosphorylation in EAhy926 cells.

15 Because Ang-1-FD-Fc-FD is a mutant molecule derived from the agonist angiopoietin-1 and Ang-2-FD-Fc-FD is a mutant molecule derived from the antagonist angiopoietin-2, we wanted to determine whether or not these two mutant molecules would retain the activity associated with the parent
20 molecule from which it was derived.

Assay system: All of the experiments described *infra* utilized the cell line EAhy926 (Edgell, C. J., et al., (1983) Proc. Natl. Acad. Sci. USA 80:3734-3737) and standard phosphorylation assays and reagents familiar to those of skill
25 in the art.

(A) Ang1*-mediated vs. Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were stimulated with either 0.1 µg/ml, 0.2 µg/ml, or 0.8 µg/ml Ang1* or Ang-1-FD-Fc-FD protein.
30 A standard phosphorylation assay revealed that Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells (Figure 9).

(B) Ability of Ang-2-FD-Fc-FD to block Ang1*-mediated Tie-2 receptor

phosphorylation in EAhy926 cells: EAhy926 cells were treated with 0.4 µg/ml of the Tie-2 agonist Ang1* and 1 µg/ml, 2 µg/ml, 4 µg/ml, 6 µg/ml, or 8 µg/ml of Ang-2-FD-Fc-FD. As shown in Figure 10, Ang-2-FD-Fc-FD is able to block Ang1* stimulation of the Tie-2 receptor when it is present in at least a 10-15 fold molar excess of Ang1*.

(C) Ability of angiopoietin-2 to block Ang1*-mediated Tie-2 receptor

phosphorylation in EAhy926 cells: To compare the blocking effects of the naturally occurring antagonist angiopoietin-2 with that of Ang-2-FD-Fc-FD, the same experiment described in (B) *supra* was performed, substituting angiopoietin-2 for Ang-2-FD-Fc-FD. The results of this experiment are shown in Figure 11. At a 20 fold molar excess, the angiopoietin-2 has not reduced the phosphorylation level to 50%. This result, coupled with the results described in (B) *supra* implies that Ang-2-FD-Fc-FD is a more potent inhibitor of Ang1*-mediated Tie-2 receptor phosphorylation than angiopoietin-2.

(D) Ability of Ang-2-FD-Fc-FD to block angiopoietin-1-mediated

phosphorylation of the Tie-2 receptor in EAhy926 cells: EAhy926 cells were treated with 0.2 µg/ml of the naturally occurring Tie-2 agonist angiopoietin-1 and 1 µg/ml, 2 µg/ml, 4 µg/ml, 6 µg/ml, or 8 µg/ml of Ang-2-FD-Fc-FD. The results of this experiment, shown in Figure 12, show that while there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, Ang-2-FD-Fc-FD seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 10 and described in (B) *supra*.

(E) Ability of angiopoietin-2 to block angiopoietin-1-mediated

phosphorylation of the Tie-2 receptor in EAhy926 cells: EAhy926 cells were treated with 0.2 µg/ml of the angiopoietin-1 and 1 µg/ml, 2 µg/ml, 4 µg/ml,

6 µg/ ml, or 8 µg/ml of angiopoietin-2. The results of this experiment, shown in Figure 13, show that there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, but, like Ang-2-FD-Fc-FD, angiopoietin-2 seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 11 and described in (C) *supra*.

Example 5: Construction of Ang-1-FD-Fc-FD CHO cell expression vector pRG763/Ang-1-FD-Fc-FD.

The pRG763/Ang-1-FD-Fc-FD CHO cell expression vector was constructed by isolating from the plasmid pCDNA3.1/Ang1-FD-Fc-FD a 2115 base pair EcoRI - NotI fragment containing Ang1-FD-Fc-FD and ligating this fragment into pRG763 vector digested with EcoRI and NotI. A large scale (2L) culture of E. coli DH10B cells carrying the pRG763/Ang-1-FD-Fc-FD plasmid was grown overnight in TB + ampicillin and the plasmid DNA was extracted using a Promega Wizard Plus Maxiprep kit, following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was verified by digestion of aliquots with NcoI and HincII restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

Example 6: Expression of Ang-1-FD-Fc-FD in CHO cells.

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 10⁶ cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 µg of pRG763/Ang-1-FD-Fc-FD using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10%

FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3
5 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and purified as described *infra*.

Example 7: Construction of Ang-2-FD-Fc-FD CHO cell expression vector
10 **pRG763/Ang-2-FD-Fc-FD.**

The plasmid pRG763/Ang-2-FD-Fc-FD was constructed by isolating from the plasmid pCDNA3.1/Ang-2-FD-Fc-FD a 2097 base pair EcoRI - NotI fragment containing Ang-2-FD-Fc-FD and ligating this fragment into the pRG763
15 vector digested with EcoRI and NotI. A large scale (1L) culture of E. coli DH10B cells carrying the pRG763/Ang-2-FD-Fc-FD plasmid was grown overnight in TB + ampicillin and the plasmid DNA was extracted using a Promega Wizard Plus Maxiprep kit, following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV
20 spectrophotometer and fluorometer. The plasmid DNA was also verified by digestion of plasmid DNA with NcoI and Ppu10I restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

25 **Example 8: Expression of Ang-2-FD-Fc-FD in CHO cells.**

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 10⁶ cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with
30 glutamine. The following day each plate was transfected with 6 µg of pRG763/Ang-2-FD-Fc-FD using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after

adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10% FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles purified as described infra.

Example 9: Characterization of stable CHO clone-derived Ang-1-FD-Fc-FD protein.

Molecular Weight Analysis: The predicted molecular weight for stable CHO clone-derived Ang-1-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form (with respect to the Fc) has a predicted weight of 76,349. In addition, there are three predicted N-linked glycosylation sites, approximately 2500 MW/site, that could potentially increase the molecular weight of the monomeric protein to 83,849. Due to the interaction between the Fc components of adjacent molecules, the protein actually exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,698. Subsequent SDS PAGE analyses confirmed these approximate molecular weights, with a band running at about 210kD under non-reducing conditions and a band running at about 85kD under reducing conditions. Light scatter analysis was performed to further confirm the molecular weight and, more importantly, determine whether or not the protein was a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent was monitored with an on line light scattering detector and a refractive index and/or a UV detector. The light scattering detector is a MiniDawn laser light scattering detector was from Wyatt Technology

Corporation (Santa Barbara, CA). This instrument measures static light at three different angles. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) was used to calculate the protein concentration based on either dn/dc (dn = change of refractive index; dc = concentration) or the extinction coefficient of the protein. The molecular weight of protein is then calculated from the angular dependence of light scattering. The results of this analysis show that the dimeric protein appears to be approximately 173.9kD and the presence of a single peak implies that the protein solution is homogenous.

Expression level of Ang-1-FD-Fc-FD in stable CHO clones: CHO cell supernatant containing recombinant Ang-1-FD-Fc-FD protein was generated by stably transfecting CHO cells with the Ang-1-FD-Fc-FD DNA construct described *supra*. The CHO cell supernatant was analyzed by standard ELISA using an anti-human IgG antibody as a capture antibody and an anti-human IgG antibody conjugated to alkaline phosphatase as a reporter antibody to quantitate the amount of Ang-1-FD-Fc-FD protein present in the supernatant. This analysis revealed expression levels of 2-3 pg/cell/day.

Purification of Ang-1-FD-Fc-FD protein derived from stable CHO clone

supernatants: Because the Ang-1-FD-Fc-FD protein contains an Fc domain, purification is relatively simple and straight forward using standard Protein A column chromatography (Pharmacia, Inc.) followed by standard size exclusion chromatography (Pharmacia, Inc.). The CHO cell supernatant was purified as described *supra* and the purified ANG-1-FD-Fc-FD protein was used in the studies described *infra* to further characterize the protein.

N-terminal sequencing of stable CHO clone-derived Ang-1-FD-Fc-FD

protein: Purified Ang-1-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal

sequence, Arg-Asp-X-Ala-Asp, wherein X is Cys. This sequence can be found at amino acids 16-20 of Figure 3A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 Figure 3A.

5 **Example 10: Characterization of stable CHO clone-derived Ang-2-FD-Fc-FD protein.**

Molecular Weight Analysis: As described for stable CHO clone-derived Ang-1-FD-Fc-FD *supra*, the predicted molecular weight for stable CHO
10 clone-derived Ang-2-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT). The monomeric form of Ang-2-FD-Fc-FD has a predicted weight of 76,052, with three predicted N-linked glycosylation sites that could potentially increase the molecular weight of the monomeric protein to 83,552. Like
15 Ang-1-FD-Fc-FD, the protein exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,104. SDS PAGE analyses confirmed these approximate molecular weights, with a band running at about 200kD under non-reducing conditions and a band running at about 85kD under reducing conditions. Light scatter analysis confirmed
20 the molecular weight (176.6kD) and revealed that the stable CHO clone-derived Ang-2-FD-Fc-FD protein, like stable CHO clone-derived Ang-1-FD-Fc-FD, exists as a homogeneous species.

Expression level of Ang-2-FD-Fc-FD derived from stable CHO clones: CHO

25 cell supernatant containing recombinant Ang-2-FD-Fc-FD protein was generated by stably transfecting CHO cells with the Ang-2-FD-Fc-FD DNA construct described *supra*. The CHO cell supernatant was analyzed by standard ELISA using an anti-human IgG antibody as a capture antibody and an anti-human IgG antibody conjugated to alkaline phosphatase as a
30 reporter antibody to quantitate the amount of Ang-2-FD-Fc-FD protein present in the supernatant. This analysis revealed expression levels of approximately 1-2 pg/cell/day.

Purification of stable CHO clone-derived Ang-2-FD-Fc-FD from cell

supernatants: As with Ang-1-FD-Fc-FD, Ang-2-FD-Fc-FD protein contains an Fc domain, so purification is relatively simple and straight forward using standard Protein A column chromatography followed by standard size exclusion chromatography. The CHO cell supernatant was purified as described for stable CHO clone-derived Ang-1-FD-Fc-FD *supra* and was used in the studies described *infra* to further characterize this protein.

N-terminal sequencing of stable CHO clone-derived Ang-2-FD-Fc-FD

protein: Purified stable CHO clone-derived Ang-2-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal sequence, Asp-X-Ala-Glu-Val, wherein X is Cys. This sequence can be found at amino acids 17-21 of Figure 4A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 of Figure 4A.

Example 11: Effects of stable CHO clone-derived Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD on Tie-2 receptor phosphorylation in EAhy926 cells.

Assay system: All of the experiments described *infra* utilized the cell line EAhy926 (Edgell, C. J., et al., (1983) Proc. Natl. Acad. Sci. USA 80:3734-3737) and standard phosphorylation assays and reagents familiar to those of skill in the art.

(A) Ang1*-mediated vs. stable CHO clone-derived Ang-1-FD-Fc-FD-

mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were stimulated with 0.4 µg/ml Ang1* or 0.2 µg/ml or 0.4 µg/ml stable CHO clone-derived Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that or stable CHO clone-derived Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells (Figure 17).

(B) Ability of stable CHO clone-derived Ang-2-FD-Fc-FD to block stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in

EAhy926 cells: EAhy926 cells were treated with 0.2 µg/ml of the Tie-2 agonist Ang-1-FD-Fc-FD and 2 µg/ml, 4 µg/ml, 8 µg/ml or 16 µg/ml of stable CHO clone-derived Ang-2-FD-Fc-FD. As shown in Figure 18, Ang-2-FD-Fc-FD is able to block stable CHO clone-derived Ang-1-FD-Fc-FD stimulation of the Tie-2 receptor when it is present in at least a 40 fold molar excess of stable CHO clone-derived Ang-1-FD-Fc-FD.

Ephrin ligands:

In previous experiments (Davis et al., 1994, Science, 266:816-819; Gale et al., 1996, Neuron 17:9-19, Gale and Yancopoulos, 1997, Cell Tissue Research 290:227-241), soluble, unclustered Ephrin-B1-Fc and Ephrin-B2-Fc, which dimerize at their respective Fc domains and therefore are dimeric with respect to either the Ephrin-B1 or Ephrin-B2 ectodomain, failed to induce EphB2 receptor phosphorylation. However, when either molecule was multimerized by pre-clustering with an anti-Fc antibody, they became potent agonists for the EphB2 receptor, as judged by tyrosine phosphorylation of the EphB2 receptor in a COS cell reporter assay. Because multimerization of both Ephrin-B1 and Ephrin-B2 appears to be necessary for induction of receptor phosphorylation, we theorized that a molecule that contained tandem repeats of either Ephrin-B1 or Ephrin-B2 ectodomains fused to an Fc domain, which would be dimeric with respect to the Fc domain but which would be tetrameric with respect to Ephrin ectodomains, might be sufficiently clustered to induce receptor phosphorylation. To test this hypothesis, the following DNA constructs were constructed, recombinant proteins produced, and reporter assays performed.

Construction of tandem Ephrin ectodomain/Fc domain nucleic acid molecules.

5 All of the following nucleic acid molecules were constructed by standard recombinant DNA techniques (See e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY), sequence-verified by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator
10 Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA), and subcloned into either the mammalian expression pJFE14 (Ephrin-B1-Ephrin-B1-Fc) or pMT21 (Ephrin-B2-Ephrin-B2-Fc), each with a Kozak sequence (Kozak, M., 1987, Nucleic Acids Res. 15:8125-8148) at the 5' end to promote protein translation. The bridging sequences described *infra* were
15 introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains, but there is no indication that there is a very critical nature to these bridging sequences (though varying the length of the linker in some of these constructs led to some variation in the amount of protein produced).

20 **Example 12: Construction of Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc nucleic acid molecules.**

(A) Ephrin-B1-Ephrin-B1-Fc: The Ephrin-B1-Ephrin-B1-Fc DNA molecule
25 consists of the coding sequence of the ectodomain of Ephrin-B1 (Davis et al., *ibid.*), which corresponds to nucleotides 1-711 of Figure 14A-Figure 14B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 712-720 of Figure 14B), followed by a second copy of the ectodomain of Ephrin-B1 (corresponding to nucleotides 721-1344 of Figure
30 14B-Figure 14D), except that in this copy of the Ephrin-B1 ectodomain the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1345-1353 of Figure 14D),

followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1354-2049 of Figure 14D-Figure 14E).

(B) Ephrin-B2-Ephrin-B2-Fc: The Ephrin-B2-Ephrin-B2-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B2 (Bergemann et al., 1995, Mol. Cell Biol. 15:4821-4929), which corresponds to nucleotides 1-675 of Figure 15A-Figure 15B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 676-684 of Figure 15B), followed by a second copy of the ectodomain of Ephrin-B2 (corresponding to nucleotides 685-1270 of Figure 15B-Figure 15D), except that in this copy the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1270-1278 of Figure 15D), followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1279-1977 of Figure 15D-Figure 15E).

As with the angiopoietin nucleic acid molecules described *supra*, the bridging sequences were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains.

Example 13: Expression of tandem Ephrin recombinant proteins in COS cells.

COS cells were transiently transfected with either the Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc nucleic acid molecules described *supra* using standard transfection techniques known in the art. Two days subsequent to transfection, the growth medium (DMEM supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, and 10% calf serum) was aspirated and replaced with serum-free medium (DMEM supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine). Cell were grown for an additional three days and then the serum-free medium containing the recombinant proteins was collected. Recombinant protein concentration was determined by performing dot blots and comparing the

signal obtained to a standard curve. Once approximate protein concentrations were determined, the Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc recombinant proteins were used in the cell reporter assays described *infra*.

5

Example 14: Characterization of the COS cell-derived tandem Ephrin ectodomain/Fc domain recombinant proteins.

Reporter Assay: COS cells, which endogenously express the Eph family receptor EphB2 (Gale et al., 1996, Neuron 17:9-19), were used in reporter assays to evaluate the ability of Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc to induce receptor phosphorylation. The assays were performed as previously described (Davis et al., *ibid.*; Gale et al., *ibid.*). Briefly, COS cells were grown to 80-90% confluency in standard growth medium described *supra*. After growth, the medium was aspirated, and replaced with serum-free medium (described *supra*) for 1-2 hours prior to treatment with either Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc recombinant protein. The cells were stimulated with 500 ng/ml Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc for 30 minutes at 37°C, with or without affinity purified human IgG1 Fc-specific goat anti-human antibody (Jackson ImmunoResearch, West Grove, PA) at a final concentration of 17 µg/ml. This antibody is capable of clustering the Fc tagged fusion. Subsequent to treatment, the COS cells were harvested and cell lysates were prepared as described in Davis, et al. and Gale, et al., *supra*. The EphB2 receptor protein was immunoprecipitated from the cell lysates using an anti-EphB2 antisera (Henkemeyer et al., 1994, Oncogene 9:1001-1014). Immunoprecipitates were resolved by standard SDS PAGE and transferred to PVDF membranes (Millipore) for western blot analysis. The membranes were probed with either anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology Institute, Lake Placid, NY) or anti-EphB2 antibodies (Henkemeyer, et al., *ibid.*) to determine the extent of EphB2

phosphorylation and the relative quantities of EphB2 in the experimental conditions described *supra*.

Results: Both Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc were shown to be approximately as active as anti-Fc antibody-clustered Ephrin-B1-Fc in their ability to induce EphB2 receptor phosphorylation in the COS cell reporter assay. Furthermore, if either of the proteins were further clustered with the goat anti-human Fc antibody, they became even more potent in their ability to induce EphB2 receptor phosphorylation. Figure 16 shows the results of this phosphorylation assay.

Example 15: Construction of Ephrin-B2-Ephrin-B2-Fc CHO expression vector.

The Ephrin-B2-Ephrin-B2-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B2 (Bergemann et al., 1995, Mol. Cell Biol. 15:4821-4929), which corresponds to nucleotides 1-675 of Figure 15A-Figure 15B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 676-684 of Figure 15B), followed by a second copy of the ectodomain of Ephrin-B2 (corresponding to nucleotides 685-1270 of Figure 15B-Figure 15D), except that in this copy the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1270-1278 of Figure 15D), followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1279-1977 of Figure 15D-Figure 15E). This molecule was subcloned into the HindIII and NotI polylinker sites in the expression vector pRG763 and was designated pRG763-m(Ephrin-B2)2-Fc. As with the angiopoietin nucleic acid molecules described *supra*, the bridging sequences were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains.

Example 16: Expression of Ephrin-B2-Ephrin-B2-Fc in CHO-K1 (E1A) cells.

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4×10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal

5 Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 μ g of pRG763-m(Ephrin-B2)2-Fc using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10%

10 FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged
15 at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and purified as described *supra*.

WHAT IS CLAIMED IS:

1. A nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of the receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, said multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.
2. The nucleic acid of claim 1, wherein the receptor binding domains of the first and second subunits are copies of the receptor binding domain of the same ligand.
3. The nucleic acid of claim 1, wherein the receptor binding domains of the first subunit are copies of the receptor binding domain of a different ligand from the receptor binding domains of the second subunit.
4. The nucleic acid of claim 2, wherein the first and second subunits each have one copy of the receptor binding domain of the ligand.
5. The nucleic acid of claim 3, wherein the first and second subunits each have one copy of the receptor binding domain of the ligand.
6. The nucleic acid of claim 2, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.
7. The nucleic acid of claim 4, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

8. The nucleic acid of claim 2, wherein the ligand is selected from the group consisting of the EPH family of ligands.
9. The nucleic acid of claim 4, wherein the ligand is selected from the group consisting of the EPH family of ligands.
10. The nucleic acid of claims 1 through 9, wherein the multimerizing component comprises an immunoglobulin derived domain.
11. The nucleic acid molecule of claim 10, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.
12. A fusion polypeptide encoded by the nucleic acid molecule of claims 1 through 11.
13. A composition comprising a multimer of the fusion polypeptide of claim 12.
14. The composition of claim 13, wherein the multimer is a dimer.
15. A vector which comprises the nucleic acid molecule of claims 1 through 11.
16. An expression vector comprising a nucleic acid molecule of claims 1 through 11, wherein the nucleic acid molecule is operatively linked to an expression control sequence.
17. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 16, in a suitable host cell.

18. The host-vector system of claim 17, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.
19. The host-vector system of claim 17, wherein the suitable host cell is E. coli.
20. The host-vector system of claim 17, wherein the suitable host cell is a COS cell.
21. The host-vector system of claim 17, wherein the suitable host cell is a CHO cell.
22. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claims 17 through 21, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.
23. A nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises more than one copy of the receptor binding domain of a ligand in tandem, and wherein either the N-terminal or the C-terminal receptor binding domain is also fused to a multimerizing component.
24. The nucleic acid of claim 23, wherein the receptor binding domains are fused contiguously.
25. The nucleic acid of claim 23, wherein the ligand is not a member of the EPH family of ligands.
26. The nucleic acid of claim 24, wherein the ligand is not a member of

the EPH family of ligands.

27. The nucleic acid of claim 23, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.
28. The nucleic acid of claim 24, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.
29. The nucleic acid of claims 23 through 28, wherein the multimerizing component comprises an immunoglobulin derived domain.
30. The nucleic acid molecule of claim 29, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.
31. A fusion polypeptide encoded by the nucleic acid molecule of claims 23 through 30.
32. A composition comprising a multimer of the fusion polypeptide of claim 31.
33. The composition of claim 32, wherein the multimer is a dimer.
34. A vector which comprises the nucleic acid molecule of claims 23 through 30.
35. An expression vector comprising a nucleic acid molecule of claims 23 through 30, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

36. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 35, in a suitable host cell.
37. The host-vector system of claim 36, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.
38. The host-vector system of claim 36, wherein the suitable host cell is E. coli.
39. The host-vector system of claim 36, wherein the suitable host cell is a COS cell.
40. The host-vector system of claim 36, wherein the suitable host cell is a CHO cell.
41. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claims 36 through 40, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.

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      10      20      30      40
*      *      *      *      *      *
ATG TCT GCA CTT CTG ATC CTA GCT CTT GTT GGA GCT GCA GTT GCT
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala>
_a_a_a_a_TRYPSIN SIGNAL SEQUENCE_a_a_a_a>

      50      60      70      80      90
*      *      *      *      *      *
AGA GAC TGT GCA GAT GTA TAT CAA GCT GGT TTT AAT AAA AGT GGA
Arg Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly>
_b_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      100     110     120     130
*      *      *      *      *      *
ATC TAC ACT ATT TAT ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG
Ile Tyr Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val>
_b_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      140     150     160     170     180
*      *      *      *      *      *
TTT TGC AAT ATG GAT GTC AAT GGG GGA GGT TGG ACT GTA ATA CAA
Phe Cys Asn Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln>
_b_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      190     200     210     220
*      *      *      *      *      *
CAT CGT GAA GAT GGA AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA
His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu>
_b_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      230     240     250     260     270
*      *      *      *      *      *
TAT AAA ATG GGT TTT GGA AAT CCC TCC GGT GAA TAT TGG CTG GGG
Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly>
_b_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      280     290     300     310
*      *      *      *      *      *
AAT GAG TTT ATT TTT GCC ATT ACC AGT CAG AGG CAG TAC ATG CTA
Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr Met Leu>
_b_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      320     330     340     350     360
*      *      *      *      *      *
AGA ATT GAG TTA ATG GAC TGG GAA GGG AAC CGA GCC TAT TCA CAG
Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln>
_b_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      370     380     390     400
*      *      *      *      *      *
TAT GAC AGA TTC CAC ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG
Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu>
_b_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      410     420     430     440     450
*      *      *      *      *      *
TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG
Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu>
_b_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

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      460      470      480      490
*      *      *      *      *      *
ATC TTA CAC GGT GCT GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC
Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp>
__b__b__b__ANG1 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

      500      510      520      530      540
*      *      *      *      *      *
AAC TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG
Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp>
__b__b__b__ANG1 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

      550      560      570      580
*      *      *      *      *      *
TTT GAT GCT TGT GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr>
__b__b__b__ANG1 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

      590      600      610      620      630
*      *      *      *      *      *
GCG GGA CAA AAC CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC
Ala Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr>
__b__b__b__ANG1 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

      640      650      660      670
*      *      *      *      *      *
TTC AAA GGG CCC AGT TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT
Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile>
__b__b__b__ANG1 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

      680      690      700      710      720
*      *      *      *      *      *
CGA CCT TTA GAT TTT GGC CCC GCG CCT TTT AGA GAC TGT GCA GAT
Arg Pro Leu Asp Phe>
__ANG1 FIBRINO____>
      Gly Pro Ala Pro>
      __GPAP BRI____>
      Phe Arg Asp Cys Ala Asp>
      __ANG1 FIBRINOGEN-____>

      730      740      750      760
*      *      *      *      *      *
GTA TAT CAA GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT TAT
Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

      770      780      790      800      810
*      *      *      *      *      *
ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT
Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

      820      830      840      850
*      *      *      *      *      *
GTC AAT GGG GGA GGT TGG ACT GTA ATA CAA CAT CGT GAA GAT GGA
Val Asn Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

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      860          870          880          890          900
      *            *            *            *            *
AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA TAT AAA ATG GGT TTT
Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

      910          920          930          940
      *            *            *            *            *
GGA AAT CCC TCC GGT GAA TAT TGG CTG GGG AAT GAG TTT ATT TTT
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

      950          960          970          980          990
      *            *            *            *            *
GCC ATT ACC AGT CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG
Ala Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

      1000         1010         1020         1030
      *            *            *            *            *
GAC TGG GAA GGG AAC CGA GCC TAT TCA CAG TAT GAC AGA TTC CAC
Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

      1040         1050         1060         1070         1080
      *            *            *            *            *
ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG TAT TTA AAA GGT CAC
Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

      1090         1100         1110         1120
      *            *            *            *            *
ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG ATC TTA CAC GGT GCT
Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

      1130         1140         1150         1160         1170
      *            *            *            *            *
GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC AAC TGT ATG TGC AAA
Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys Met Cys Lys>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

      1180         1190         1200         1210
      *            *            *            *            *
TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG TTT GAT GCT TGT GGC
Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

      1220         1230         1240         1250         1260
      *            *            *            *            *
CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA AAC CAT
Pro Asn Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn His>
__d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

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	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390	2391	2392	2393	2394	2395	2396	2397	2398	2399	2400	2401	2402	2403	2404	2405	2406	2407	2408	2409	2410	2411	2412	2413	2414	2415	2416	2417	2418	2419	2420	2421	2422	2
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1270      1280      1290      1300
*         *         *         *         *
GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCC AGT
Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser>
_d_d_d_ANG1 FIBRINOGEN-LIKE DOMAIN_d_d_d_d_>

1310      1320      1330      1340      1350
*         *         *         *         *
TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT
Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe>
_d_d_d_ANG1 FIBRINOGEN-LIKE DOMAIN_d_d_d_d_>

1360      1370      1380      1390
*         *         *         *         *
GGA CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA
Gly Pro Gly>
_e_e_>
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro>
_f_f_f_FC TAG [SPLIT]_f_f_f_f_>

1400      1410      1420      1430      1440
*         *         *         *         *
CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu>
_f_f_f_f_f_FC TAG [SPLIT]_f_f_f_f_f_>

1450      1460      1470      1480
*         *         *         *         *
TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro>
_f_f_f_f_f_FC TAG [SPLIT]_f_f_f_f_f_>

1490      1500      1510      1520      1530
*         *         *         *         *
GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu>
_f_f_f_f_f_FC TAG [SPLIT]_f_f_f_f_f_>

1540      1550      1560      1570
*         *         *         *         *
GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala>
_f_f_f_f_f_FC TAG [SPLIT]_f_f_f_f_f_>

1580      1590      1600      1610      1620
*         *         *         *         *
AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val>
_f_f_f_f_f_FC TAG [SPLIT]_f_f_f_f_f_>

1630      1640      1650      1660
*         *         *         *         *
GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys>
_f_f_f_f_f_FC TAG [SPLIT]_f_f_f_f_f_>

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Figure 1E

1670 1680 1690 1700 1710
* * * * *
GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile>
__f__f__f__f__f__FC TAG [SPLIT]__f__f__f__f__f__>

1720 1730 1740 1750
* * * * *
GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln>
__f__f__f__f__f__FC TAG [SPLIT]__f__f__f__f__f__>

1760 1770 1780 1790 1800
* * * * *
GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln>
__f__f__f__f__f__FC TAG [SPLIT]__f__f__f__f__f__>

1810 1820 1830 1840
* * * * *
GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>
__f__f__f__f__f__FC TAG [SPLIT]__f__f__f__f__f__>

1850 1860 1870 1880 1890
* * * * *
GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys>
__f__f__f__f__f__FC TAG [SPLIT]__f__f__f__f__f__>

1900 1910 1920 1930
* * * * *
ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr>
__f__f__f__f__f__FC TAG [SPLIT]__f__f__f__f__f__>

1940 1950 1960 1970 1980
* * * * *
AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val>
__f__f__f__f__f__FC TAG [SPLIT]__f__f__f__f__f__>

1990 2000 2010 2020
* * * * *
TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr>
__f__f__f__f__f__FC TAG [SPLIT]__f__f__f__f__f__>

2030 2040 2050
* * * * *
CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>
__f__f__f__f__f__FC TAG [SPLIT]__f__f__f__f__f__>

0556067-100104
TOTOT 222222

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      10      20      30      40
*      *      *      *      *
ATG TCT GCA CTT CTG ATC CTA GCT CTT GTT GGA GCT GCA GTT GCT
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala>
_a_a_a_a_TRYPSIN SIGNAL SEQUENCE_a_a_a_a>

      50      60      70      80      90
*      *      *      *      *
AGA GAC TGT GCT GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC
Arg Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly>
_b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1_b_b_b_>

      100     110     120     130
*      *      *      *      *
ATC TAC ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC
Ile Tyr Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala>
_b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1_b_b_b_>

      140     150     160     170     180
*      *      *      *      *
TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG
Tyr Cys Asp Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln>
_b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1_b_b_b_>

      190     200     210     220
*      *      *      *      *
CGA CGT GAG GAT GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA
Arg Arg Glu Asp Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu>
_b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1_b_b_b_>

      230     240     250     260     270
*      *      *      *      *
TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA
Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly>
_b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1_b_b_b_>

      280     290     300     310
*      *      *      *      *
AAT GAG TTT GTT TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT
Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg Tyr Val Leu>
_b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1_b_b_b_>

      320     330     340     350     360
*      *      *      *      *
AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG
Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser Leu>
_b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1_b_b_b_>

      370     380     390     400
*      *      *      *      *
TAT GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT
Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile>
_b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1_b_b_b_>

      410     420     430     440     450
*      *      *      *      *
CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC
His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile>
_b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1_b_b_b_>

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Figure 2B

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      460      470      480      490
      *      *      *      *      *
AGC CAA CCA GGA AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC
Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN #1__b__b__b__>

      500      510      520      530      540
      *      *      *      *      *
AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG
Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN #1__b__b__b__>

      550      560      570      580
      *      *      *      *      *
TTT GAT GCA TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN #1__b__b__b__>

      590      600      610      620      630
      *      *      *      *      *
CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC
Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN #1__b__b__b__>

      640      650      660      670
      *      *      *      *      *
TGG AAA GGC TCA GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC
Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN #1__b__b__b__>

      680      690      700      710      720
      *      *      *      *      *
CGA CCA GCA GAT TTC GGG GGC CCC GCG CCT TTC AGA GAC TGT GCT
Arg Pro Ala Asp Phe>
__ANG2 FIBRINO__>
      Gly Gly Pro Ala Pro>
      __GGPAP BRIDGE__>
      Phe Arg Asp Cys Ala>
      __ANG2 FIBRINO__>

      730      740      750      760
      *      *      *      *      *
GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC ATC TAC ACG TTA
Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu>
__d__d__d__ANG2 FIBRINOGEN-LIKE DOMAIN#2__d__d__d__>

      770      780      790      800      810
      *      *      *      *      *
ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG
Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met>
__d__d__d__ANG2 FIBRINOGEN-LIKE DOMAIN#2__d__d__d__>

      820      830      840      850
      *      *      *      *      *
GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT
Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp>
__d__d__d__ANG2 FIBRINOGEN-LIKE DOMAIN#2__d__d__d__>

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090667-100404
 100404-090667

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      860          870          880          890          900
      *            *            *            *            *
GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA
Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

      910          920          930          940
      *            *            *            *            *
TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA AAT GAG TTT GTT
Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

      950          960          970          980          990
      *            *            *            *            *
TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT AAA ATA CAC CTT
Ser Gln Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

     1000          1010          1020          1030
      *            *            *            *            *
AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG TAT GAA CAT TTC
Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

     1040          1050          1060          1070          1080
      *            *            *            *            *
TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA
Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

     1090          1100          1110          1120
      *            *            *            *            *
CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC AGC CAA CCA GGA
Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

     1130          1140          1150          1160          1170
      *            *            *            *            *
AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC AAA TGT ATT TGC
Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys Cys Ile Cys>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

     1180          1190          1200          1210
      *            *            *            *            *
AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG TTT GAT GCA TGT
Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

     1220          1230          1240          1250          1260
      *            *            *            *            *
GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG AGG CAG AAC
Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln Asn>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

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Figure 2D

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1270      1280      1290      1300
*         *         *         *
ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC TGG AAA GGC TCA
Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

1310      1320      1330      1340      1350
*         *         *         *         *
GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC CGA CCA GCA GAT
Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp>
_d_d_d_ANG2 FIBRINOGEN-LIKE DOMAIN#2_d_d_d_>

1360      1370      1380      1390
*         *         *         *         *
TTC GGA CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC
Phe>
____>
  Gly Pro Gly>
  _e_e_>
    Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys>
    _f_f_f_f_FC TAG_f_f_f_f_f_>

1400      1410      1420      1430      1440
*         *         *         *         *
CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe>
_f_f_f_f_f_f_FC TAG_f_f_f_f_f_f_f_>

1450      1460      1470      1480
*         *         *         *         *
CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr>
_f_f_f_f_f_f_FC TAG_f_f_f_f_f_f_f_>

1490      1500      1510      1520      1530
*         *         *         *         *
CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro>
_f_f_f_f_f_f_FC TAG_f_f_f_f_f_f_f_>

1540      1550      1560      1570
*         *         *         *         *
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn>
_f_f_f_f_f_f_FC TAG_f_f_f_f_f_f_f_>

1580      1590      1600      1610      1620
*         *         *         *         *
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg>
_f_f_f_f_f_f_FC TAG_f_f_f_f_f_f_f_>

1630      1640      1650      1660
*         *         *         *         *
GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly>
_f_f_f_f_f_f_FC TAG_f_f_f_f_f_f_f_>

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1670          1680          1690          1700          1710
*            *            *            *            *
AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro>
__f__f__f__f__f__f__FC TAG__f__f__f__f__f__f__>

          1720          1730          1740          1750
*            *            *            *            *
ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro>
__f__f__f__f__f__f__FC TAG__f__f__f__f__f__f__>

    1760          1770          1780          1790          1800
*            *            *            *            *
CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn>
__f__f__f__f__f__f__FC TAG__f__f__f__f__f__f__>

          1810          1820          1830          1840
*            *            *            *            *
CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp>
__f__f__f__f__f__f__FC TAG__f__f__f__f__f__f__>

    1850          1860          1870          1880          1890
*            *            *            *            *
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr>
__f__f__f__f__f__f__FC TAG__f__f__f__f__f__f__>

          1900          1910          1920          1930
*            *            *            *            *
AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu>
__f__f__f__f__f__f__FC TAG__f__f__f__f__f__f__>

    1940          1950          1960          1970          1980
*            *            *            *            *
TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn>
__f__f__f__f__f__f__FC TAG__f__f__f__f__f__f__>

          1990          2000          2010          2020
*            *            *            *            *
GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr>
__f__f__f__f__f__f__FC TAG__f__f__f__f__f__f__>

    2030          2040          2050          2060
*            *            *            *            *
ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>
__f__f__f__f__f__f__FC TAG__f__f__f__f__f__f__>

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[illegible]

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      10      20      30      40
*      *      *      *      *      *
ATG TCT GCA CTT CTG ATC CTA GCT CTT GTT GGA GCT GCA GTT GCT
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala>
_a_a_a_a_TRYPSIN SIGNAL SEQUENCE_a_a_a_a>

      50      60      70      80      90
*      *      *      *      *      *
AGA GAC TGT GCA GAT GTA TAT CAA GCT GGT TTT AAT AAA AGT GGA
Arg Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly>
_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      100     110     120     130
*      *      *      *      *      *
ATC TAC ACT ATT TAT ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG
Ile Tyr Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val>
_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      140     150     160     170     180
*      *      *      *      *      *
TTT TGC AAT ATG GAT GTC AAT GGG GGA GGT TGG ACT GTA ATA CAA
Phe Cys Asn Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln>
_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      190     200     210     220
*      *      *      *      *      *
CAT CGT GAA GAT GGA AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA
His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu>
_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      230     240     250     260     270
*      *      *      *      *      *
TAT AAA ATG GGT TTT GGA AAT CCC TCC GGT GAA TAT TGG CTG GGG
Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly>
_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      280     290     300     310
*      *      *      *      *      *
AAT GAG TTT ATT TTT GCC ATT ACC AGT CAG AGG CAG TAC ATG CTA
Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr Met Leu>
_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      320     330     340     350     360
*      *      *      *      *      *
AGA ATT GAG TTA ATG GAC TGG GAA GGG AAC CGA GCC TAT TCA CAG
Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln>
_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

      370     380     390     400
*      *      *      *      *      *
TAT GAC AGA TTC CAC ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG
Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu>
_b_b_b_ANG1 FIBRINOGEN-LIKE DOMAIN_b_b_b_b>

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Figure 3B

410 420 430 440 450
 * * * * *
 TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG
 Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu>
 ___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

460 470 480 490
 * * * * *
 ATC TTA CAC GGT GCT GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC
 Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp>
 ___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

500 510 520 530 540
 * * * * *
 AAC TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG
 Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp>
 ___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

550 560 570 580
 * * * * *
 TTT GAT GCT TGT GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT
 Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr>
 ___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

590 600 610 620 630
 * * * * *
 GCG GGA CAA AAC CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC
 Ala Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr>
 ___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

640 650 660 670
 * * * * *
 TTC AAA GGG CCA AGT TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT
 Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile>
 ___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

680 690 700 710 720
 * * * * *
 CGA CCT TTA GAT TTT GGC CCG GGC GAG CCC AAA TCT TGT GAC AAA
 Arg Pro Leu Asp Phe>
 ___ANG1 FIBRINO___>
 Gly Pro Gly>
 ___c___c___>
 Glu Pro Lys Ser Cys Asp Lys>
 ___d___d___FC TAG___d___d___>

730 740 750 760
 * * * * *
 ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA
 Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly>
 ___d___d___d___d___d___d___FC TAG___d___d___d___d___d___d___>

770 780 790 800 810
 * * * * *
 CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met>
 ___d___d___d___d___d___d___FC TAG___d___d___d___d___d___d___>

TTTGGT 42339650

820 830 840 850

* * * * *

ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

860 870 880 890 900

* * * * *

CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

910 920 930 940

* * * * *

GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

950 960 970 980 990

* * * * *

AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

1000 1010 1020 1030

* * * * *

TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

1040 1050 1060 1070 1080

* * * * *

CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

1090 1100 1110 1120

* * * * *

CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

1130 1140 1150 1160 1170

* * * * *

CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

1180 1190 1200 1210

* * * * *

TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

1220 1230 1240 1250 1260

* * * * *

GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

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Figure 3D

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      1270      1280      1290      1300
      *      *      *      *      *      *      *      *
TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

      1310      1320      1330      1340      1350
      *      *      *      *      *      *      *      *
CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

      1360      1370      1380      1390
      *      *      *      *      *      *      *      *
CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

      1400      1410      1420      1430      1440
      *      *      *      *      *      *      *      *
GGC GGT GGC GGT TCT GGC GCG CCT TTT AGA GAC TGT GCA GAT GTA
Gly Gly Gly Gly Ser Gly Ala Pro>
_G4S LINKER/ASC BRIDGE (N____>
                                Phe Arg Asp Cys Ala Asp Val>
                                _ANG1 FIBRINOGEN-LIKE____>

      1450      1460      1470      1480
      *      *      *      *      *      *      *      *
TAT CAA GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT TAT ATT
Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile>
_f_f_f_f_ANG1 FIBRINOGEN-LIKE DOMAIN_f_f_f_f_f_f_f_f>

      1490      1500      1510      1520      1530
      *      *      *      *      *      *      *      *
AAT AAT ATG CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT GTC
Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val>
_f_f_f_f_ANG1 FIBRINOGEN-LIKE DOMAIN_f_f_f_f_f_f_f_f>

      1540      1550      1560      1570
      *      *      *      *      *      *      *      *
AAT GGG GGA GGT TGG ACT GTA ATA CAA CAT CGT GAA GAT GGA AGT
Asn Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser>
_f_f_f_f_ANG1 FIBRINOGEN-LIKE DOMAIN_f_f_f_f_f_f_f_f>

      1580      1590      1600      1610      1620
      *      *      *      *      *      *      *      *
CTA GAT TTC CAA AGA GGC TGG AAG GAA TAT AAA ATG GGT TTT GGA
Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly>
_f_f_f_f_ANG1 FIBRINOGEN-LIKE DOMAIN_f_f_f_f_f_f_f_f>

      1630      1640      1650      1660
      *      *      *      *      *      *      *      *
AAT CCC TCC GGT GAA TAT TGG CTG GGG AAT GAG TTT ATT TTT GCC
Asn Pro Ser Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala>
_f_f_f_f_ANG1 FIBRINOGEN-LIKE DOMAIN_f_f_f_f_f_f_f_f>

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Figure 3E

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1670      1680      1690      1700      1710
*         *         *         *         *
ATT ACC AGT CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG GAC
Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp>
__f__f__f__ANG1 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

      1720      1730      1740      1750
*         *         *         *         *
TGG GAA GGG AAC CGA GCC TAT TCA CAG TAT GAC AGA TTC CAC ATA
Trp Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile>
__f__f__f__ANG1 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

      1760      1770      1780      1790      1800
*         *         *         *         *
GGA AAT GAA AAG CAA AAC TAT AGG TTG TAT TTA AAA GGT CAC ACT
Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr>
__f__f__f__ANG1 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

      1810      1820      1830      1840
*         *         *         *         *
GGG ACA GCA GGA AAA CAG AGC AGC CTG ATC TTA CAC GGT GCT GAT
Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala Asp>
__f__f__f__ANG1 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

      1850      1860      1870      1880      1890
*         *         *         *         *
TTC AGC ACT AAA GAT GCT GAT AAT GAC AAC TGT ATG TGC AAA TGT
Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys Met Cys Lys Cys>
__f__f__f__ANG1 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

      1900      1910      1920      1930
*         *         *         *         *
GCC CTC ATG TTA ACA GGA GGA TGG TGG TTT GAT GCT TGT GGC CCC
Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro>
__f__f__f__ANG1 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

      1940      1950      1960      1970      1980
*         *         *         *         *
TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA AAC CAT GGA
Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn His Gly>
__f__f__f__ANG1 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

      1990      2000      2010      2020
*         *         *         *         *
AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCA AGT TAC
Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser Tyr>
__f__f__f__ANG1 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

      2030      2040      2050      2060
*         *         *         *         *
TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT
Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe>
__f__f__f__ANG1 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

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Figure 4A

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      10      20      30      40
*      *      *      *      *
ATG TCT GCA CTT CTG ATC CTA GCT CTT GTT GGA GCT GCA GTT GCT
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala>
__a__a__a__a__TRYPSIN SIGNAL SEQUENCE__a__a__a__a__>

      50      60      70      80      90
*      *      *      *      *
AGA GAC TGT GCT GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC
Arg Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN__b__b__b__b__>

      100     110     120     130
*      *      *      *      *
ATC TAC ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC
Ile Tyr Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN__b__b__b__b__>

      140     150     160     170     180
*      *      *      *      *
TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG
Tyr Cys Asp Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN__b__b__b__b__>

      190     200     210     220
*      *      *      *      *
CGA CGT GAG GAT GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA
Arg Arg Glu Asp Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN__b__b__b__b__>

      230     240     250     260     270
*      *      *      *      *
TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA
Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN__b__b__b__b__>

      280     290     300     310
*      *      *      *      *
AAT GAG TTT GTT TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT
Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg Tyr Val Leu>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN__b__b__b__b__>

      320     330     340     350     360
*      *      *      *      *
AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG
Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser Leu>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN__b__b__b__b__>

      370     380     390     400
*      *      *      *      *
TAT GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT
Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN__b__b__b__b__>

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Figure 4B

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410      420      430      440      450
*        *        *        *        *
CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC
His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

      460      470      480      490
*        *        *        *        *
AGC CAA CCA GGA AAT GAT TTT AGC ACA AAG GAT GGA AAC GAC
Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

500      510      520      530      540
*        *        *        *        *
AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG
Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

      550      560      570      580
*        *        *        *        *
TTT GAT GCA TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

590      600      610      620      630
*        *        *        *        *
CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC
Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

      640      650      660      670
*        *        *        *        *
TGG AAA GGC TCA GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC
Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile>
__b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN_b__b__b__b__>

680      690      700      710      720
*        *        *        *        *
CGA CCA GCA GAT TTC GGG GGC CCG GGC GAG CCC AAA TCT TGT GAC
Arg Pro Ala Asp Phe>
__ANG2 FIBRINO__>
      Gly Gly Pro Gly>
      __GGPG BRI__>
      Glu Pro Lys Ser Cys Asp>
      __d__FC TAG_d__d__>

      730      740      750      760
*        *        *        *        *
AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly>
__d__d__d__d__d__d__FC TAG__d__d__d__d__d__d__>

770      780      790      800      810
*        *        *        *        *
GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu>
__d__d__d__d__d__d__FC TAG__d__d__d__d__d__d__>

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Figure 4C

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      820      830      840      850
      *      *      *      *      *
ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val>
_d_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d_d_d_>

      860      870      880      890      900
      *      *      *      *      *
AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>
_d_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d_d_d_>

      910      920      930      940
      *      *      *      *      *
GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr>
_d_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d_d_d_>

      950      960      970      980      990
      *      *      *      *      *
AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln>
_d_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d_d_d_>

      1000     1010     1020     1030
      *      *      *      *      *
GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys>
_d_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d_d_d_>

      1040     1050     1060     1070     1080
      *      *      *      *      *
GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly>
_d_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d_d_d_>

      1090     1100     1110     1120
      *      *      *      *      *
CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp>
_d_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d_d_d_>

      1130     1140     1150     1160     1170
      *      *      *      *      *
GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly>
_d_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d_d_d_>

      1180     1190     1200     1210
      *      *      *      *      *
TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln>
_d_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d_d_d_>

      1220     1230     1240     1250     1260
      *      *      *      *      *
CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp>
_d_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d_d_d_>

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1270      1280      1290      1300
*         *         *         *         *         *         *         *
GGC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

1310      1320      1330      1340      1350
*         *         *         *         *         *         *         *
TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

1360      1370      1380      1390
*         *         *         *         *         *         *         *
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly>
_d_d_d_d_d_d_d_FC TAG_d_d_d_d_d_d_d_d>

1400      1410      1420      1430      1440
*         *         *         *         *         *         *         *
AAA GGC GGT GGC GGT TCT GGC GCG CCT AGA GAC TGT GCT GAA GTA
Lys>
____>
Gly Gly Gly Gly Ser Gly Ala Pro>
__e__GGGGSGAP BRIDGE_e__e__>
Arg Asp Cys Ala Glu Val>
__ANG2 FIBRINOGEN-____>

1450      1460      1470      1480
*         *         *         *         *         *         *         *
TTC AAA TCA GGA CAC ACC ACA AAT GGC ATC TAC ACG TTA ACA TTC
Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

1490      1500      1510      1520      1530
*         *         *         *         *         *         *         *
CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG GAA GCT
Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

1540      1550      1560      1570
*         *         *         *         *         *         *         *
GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT GGC AGC
Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

1580      1590      1600      1610      1620
*         *         *         *         *         *         *         *
GTT GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA TTT GGT
Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

1630      1640      1650      1660
*         *         *         *         *         *         *         *
AAC CCT TCA GGA GAA TAT TGG CTG GGA AAT GAG TTT GTT TCG CAA
Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

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1670          1680          1690          1700          1710
  *            *            *            *            *
CTG ACT AAT CAG CAA CGC TAT GTG CTT AAA ATA CAC CTT AAA GAC
Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

          1720          1730          1740          1750
  *            *            *            *            *
TGG GAA GGG AAT GAG GCT TAC TCA TTG TAT GAA CAT TTC TAT CTC
Trp Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

          1760          1770          1780          1790          1800
  *            *            *            *            *
TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA CTT ACA
Ser Ser Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly Leu Thr>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

          1810          1820          1830          1840
  *            *            *            *            *
GGG ACA GCC GGC AAA ATA AGC AGC ATC AGC CAA CCA GGA AAT GAT
Gly Thr Ala Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly Asn Asp>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

          1850          1860          1870          1880          1890
  *            *            *            *            *
TTT AGC ACA AAG GAT GGA GAC AAC GAC AAA TGT ATT TGC AAA TGT
Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys Cys Ile Cys Lys Cys>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

          1900          1910          1920          1930
  *            *            *            *            *
TCA CAA ATG CTA ACA GGA GGC TGG TGG TTT GAT GCA TGT GGT CCT
Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

          1940          1950          1960          1970          1980
  *            *            *            *            *
TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG AGG CAG AAC ACA AAT
Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln Asn Thr Asn>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

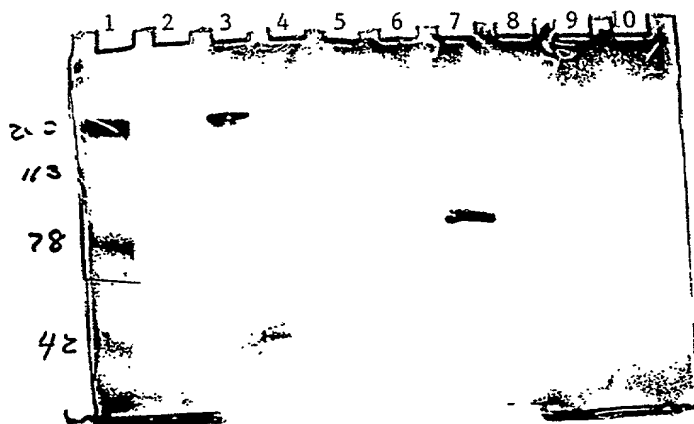
          1990          2000          2010          2020
  *            *            *            *            *
AAG TTC AAC GGC ATT AAA TGG TAC TAC TGG AAA GGC TCA GGC TAT
Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser Gly Tyr>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

          2030          2040          2050          2060          2070
  *            *            *            *            *
TCG CTC AAG GCC ACA ACC ATG ATG ATC CGA CCA GCA GAT TTC TGA
Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe>
__f__f__f__ANG2 FIBRINOGEN-LIKE DOMAIN_f__f__f__f__>

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Figure 5

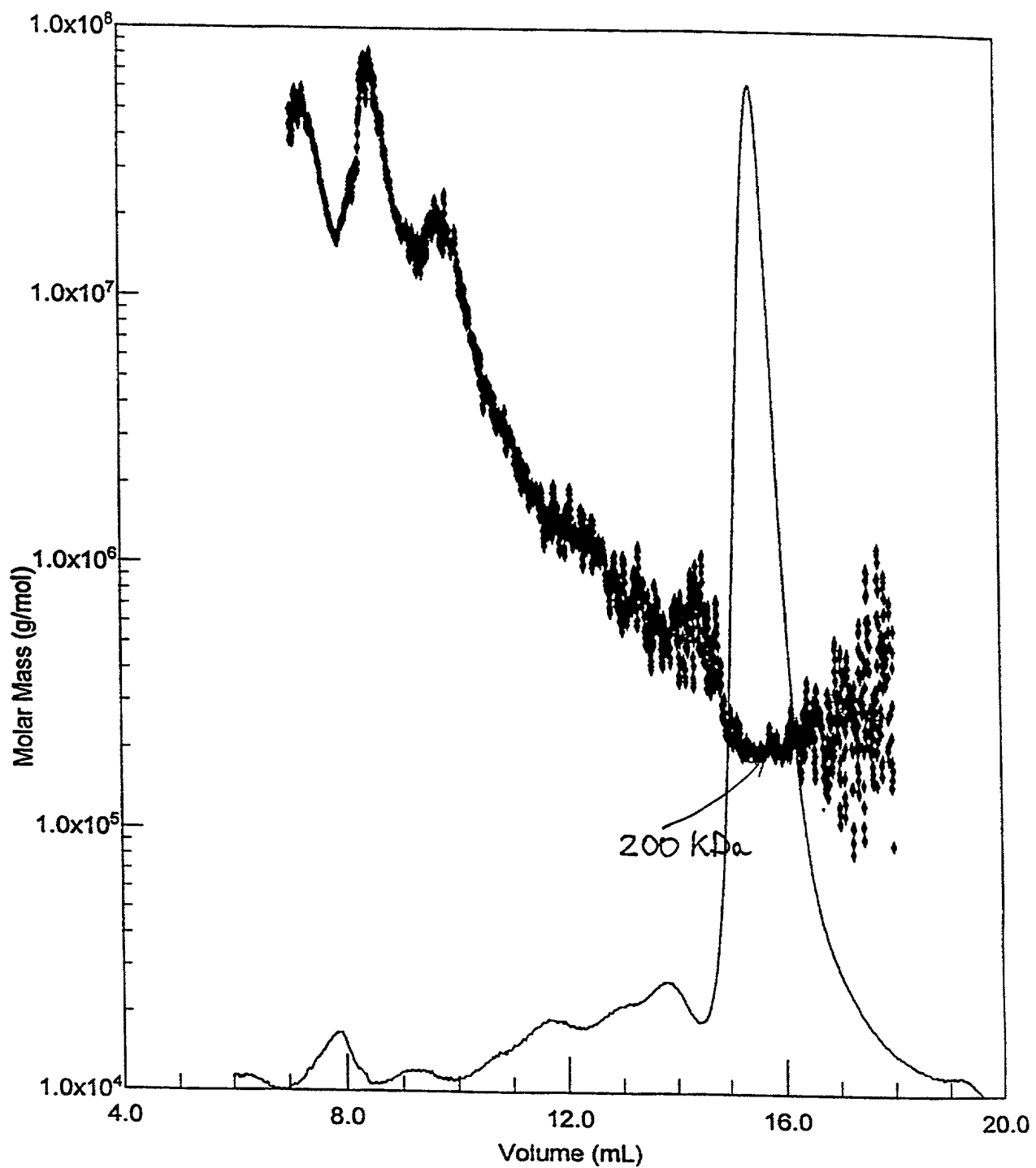


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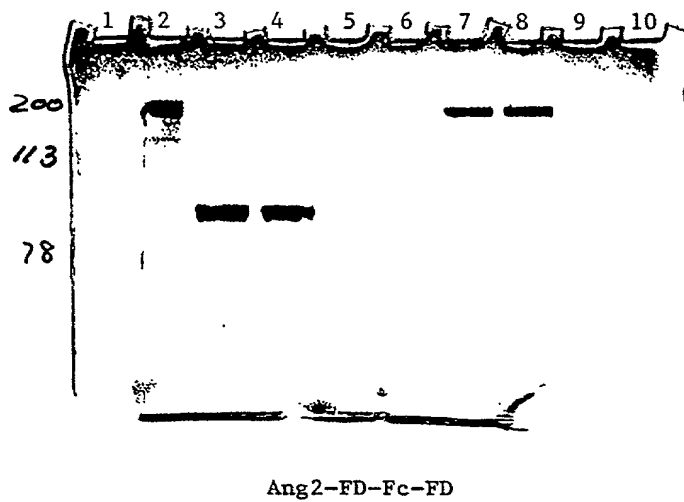
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Figure 6
Molar Mass vs. Volume



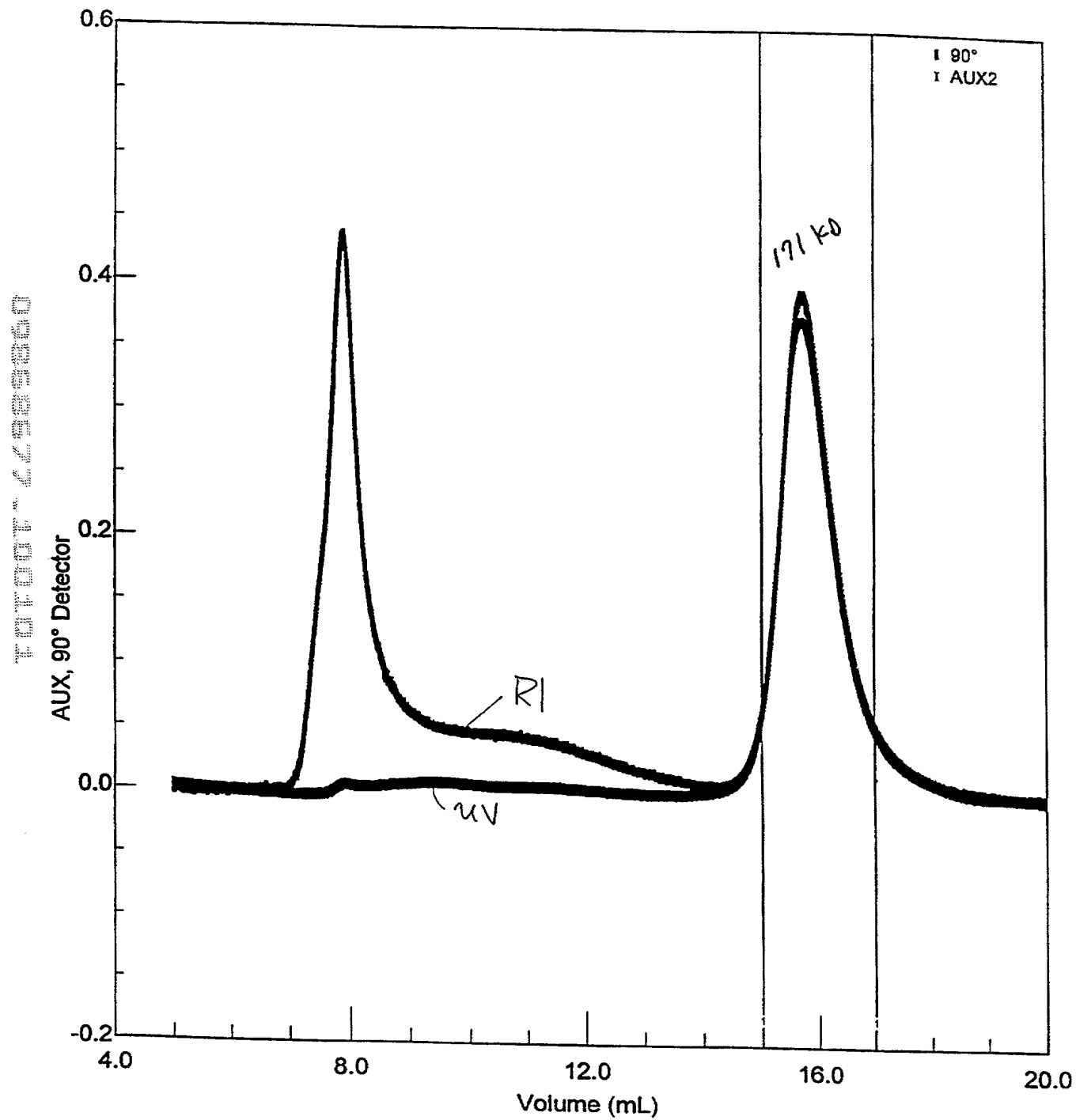
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Figure 7



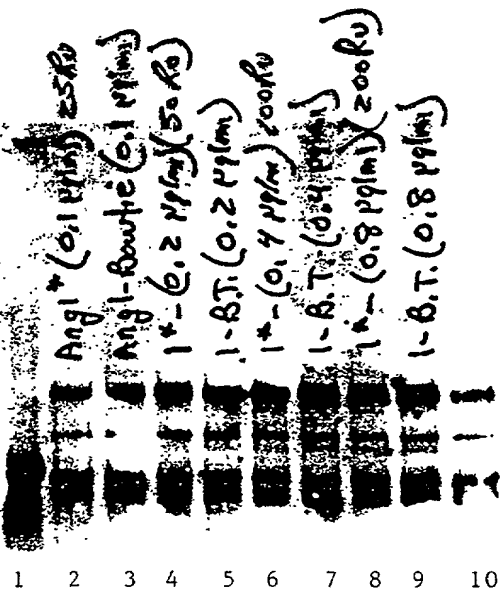
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Figure 8



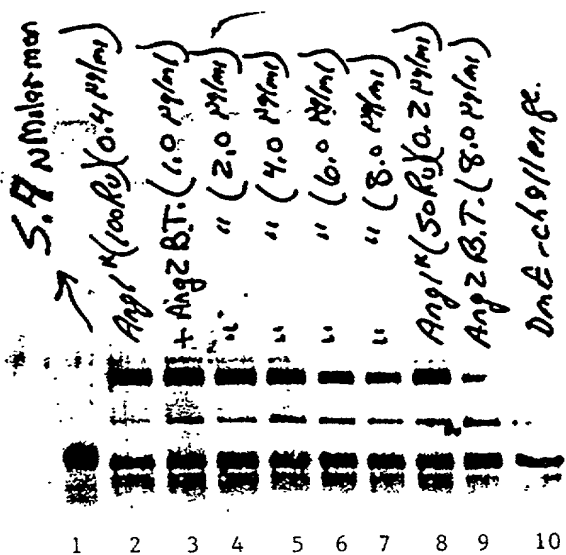
25/42

Figure 9



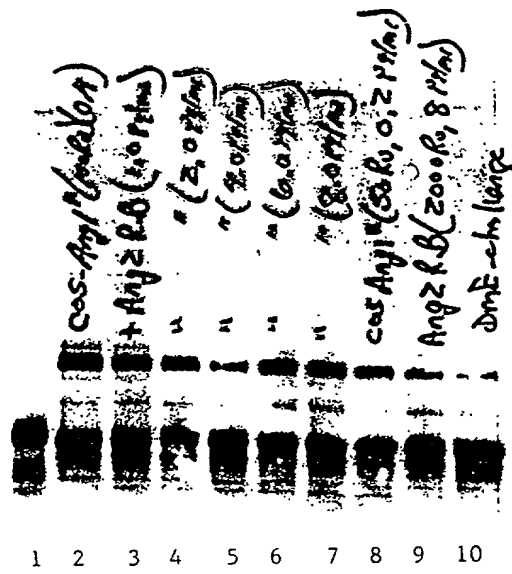
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Figure 10



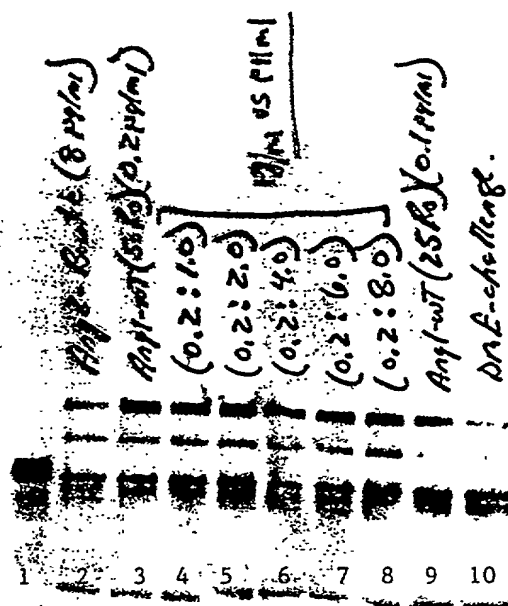
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Figure 11



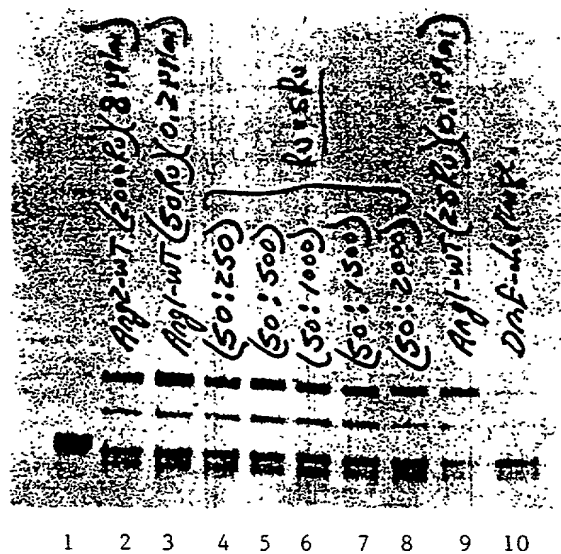
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Figure 12



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Figure 13



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Figure 14A

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      10      20      30      40
      *      *      *      *
ATG GCT CGG CCT GGG CAG CGT TGG CTC GGC AAG TGG CTT GTG GCG
Met Ala Arg Pro Gly Gln Arg Trp Leu Gly Lys Trp Leu Val Ala>
__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__>

      50      60      70      80      90
      *      *      *      *      *
ATG GTC GTG TGG GCG CTG TGC CGG CTC GCC ACA CCG CTG GCC AAG
Met Val Val Trp Ala Leu Cys Arg Leu Ala Thr Pro Leu Ala Lys>
__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__>

     100     110     120     130
      *      *      *      *      *
AAC CTG GAG CCC GTA TCC TGG AGC TCC CTC AAC CCC AAG TTC CTG
Asn Leu Glu Pro Val Ser Trp Ser Leu Asn Pro Lys Phe Leu>
__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__>

     140     150     160     170     180
      *      *      *      *      *
AGT GGG AAG GGC TTG GTG ATC TAT CCG AAA ATT GGA GAC AAG CTG
Ser Gly Lys Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu>
__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__>

     190     200     210     220
      *      *      *      *      *
GAC ATC ATC TGC CCC CGA GCA GAA GCA GGG CGG CCC TAT GAG TAC
Asp Ile Ile Cys Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr>
__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__>

     230     240     250     260     270
      *      *      *      *      *
TAC AAG CTG TAC CTG GTG CGG CCT GAG CAG GCA GCT GCC TGT AGC
Tyr Lys Leu Tyr Leu Val Arg Pro Glu Gln Ala Ala Cys Ser>
__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__>

     280     290     300     310
      *      *      *      *      *
ACA GTT CTC GAC CCC AAC GTG TTG GTC ACC TGC AAT AGG CCA GAG
Thr Val Leu Asp Pro Asn Val Leu Val Thr Cys Asn Arg Pro Glu>
__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__>

     320     330     340     350     360
      *      *      *      *      *
CAG GAA ATA CGC TTT ACC ATC AAG TTC CAG GAG TTC AGC CCC AAC
Gln Glu Ile Arg Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro Asn>
__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__>

     370     380     390     400
      *      *      *      *      *
TAC ATG GGC CTG GAG TTC AAG AAG CAC CAT GAT TAC TAC ATT ACC
Tyr Met Gly Leu Glu Phe Lys Lys His His Asp Tyr Tyr Ile Thr>
__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__>

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Figure 14B

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410          420          430          440          450
*            *            *            *            *
TCA ACA TCC AAT GGA AGC CTG GAG GGG CTG GAA AAC CGG GAG GGC
Ser Thr Ser Asn Gly Ser Leu Glu Gly Leu Glu Asn Arg Glu Gly>
__a__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__a__>

          460          470          480          490
*            *            *            *            *
GGT GTG TGC CGC ACA CGC ACC ATG AAG ATC ATC ATG AAG GTT GGG
Gly Val Cys Arg Thr Arg Thr Met Lys Ile Ile Met Lys Val Gly>
__a__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__a__>

          500          510          520          530          540
*            *            *            *            *
CAA GAT CCC AAT GCT GTG ACG CCT GAG CAG CTG ACT ACC AGC AGG
Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu Thr Thr Ser Arg>
__a__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__a__>

          550          560          570          580
*            *            *            *            *
CCC AGC AAG GAG GCA GAC AAC ACT GTC AAG ATG GCC ACA CAG GCC
Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala Thr Gln Ala>
__a__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__a__>

          590          600          610          620          630
*            *            *            *            *
CCT GGT AGT CGG GGC TCC CTG GGT GAC TCT GAT GGC AAG CAT GAG
Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys His Glu>
__a__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__a__>

          640          650          660          670
*            *            *            *            *
ACT GTG AAC CAG GAA GAG AAG AGT GGC CCA GGT GCA AGT GGG GGC
Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly Gly>
__a__a__a__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a__a__a__>

          680          690          700          710          720
*            *            *            *            *
AGC AGC GGG GAC CCT GAT GGC TTC TTC AAC TCC AAG GGC CCG GGT
Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys>
__ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)____>
                                     Gly Pro Gly>
                                     __b__b__b__>

          730          740          750          760
*            *            *            *            *
AAG AAC CTG GAG CCC GTA TCC TGG AGC TCC CTC AAC CCC AAG TTC
Lys Asn Leu Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

          770          780          790          800          810
*            *            *            *            *
CTG AGT GGG AAG GGC TTG GTG ATC TAT CCG AAA ATT GGA GAC AAG
Leu Ser Gly Lys Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

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Figure 14C

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      820      830      840      850
      *      *      *      *      *
CTG GAC ATC ATC TGC CCC CGA GCA GAA GCA GGG CGG CCC TAT GAG
Leu Asp Ile Ile Cys Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

      860      870      880      890      900
      *      *      *      *      *
TAC TAC AAG CTG TAC CTG GTG CGG CCT GAG CAG GCA GCT GCC TGT
Tyr Tyr Lys Leu Tyr Leu Val Arg Pro Glu Gln Ala Ala Cys>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

      910      920      930      940
      *      *      *      *      *
AGC ACA GTT CTC GAC CCC AAC GTG TTG GTC ACC TGC AAT AGG CCA
Ser Thr Val Leu Asp Pro Asn Val Leu Val Thr Cys Asn Arg Pro>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

      950      960      970      980      990
      *      *      *      *      *
GAG CAG GAA ATA CGC TTT ACC ATC AAG TTC CAG GAG TTC AGC CCC
Glu Gln Glu Ile Arg Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

     1000     1010     1020     1030
      *      *      *      *      *
AAC TAC ATG GGC CTG GAG TTC AAG AAG CAC CAT GAT TAC TAC ATT
Asn Tyr Met Gly Leu Glu Phe Lys Lys His His Asp Tyr Tyr Ile>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

     1040     1050     1060     1070     1080
      *      *      *      *      *
ACC TCA ACA TCC AAT GGA AGC CTG GAG GGG CTG GAA AAC CGG GAG
Thr Ser Thr Ser Asn Gly Ser Leu Glu Gly Leu Glu Asn Arg Glu>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

     1090     1100     1110     1120
      *      *      *      *      *
GGC GGT GTG TGC CGC ACA CGC ACC ATG AAG ATC ATC ATG AAG GTT
Gly Gly Val Cys Arg Thr Arg Thr Met Lys Ile Ile Met Lys Val>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

     1130     1140     1150     1160     1170
      *      *      *      *      *
GGG CAA GAT CCC AAT GCT GTG ACG CCT GAG CAG CTG ACT ACC AGC
Gly Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu Thr Thr Ser>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

     1180     1190     1200     1210
      *      *      *      *      *
AGG CCC AGC AAG GAG GCA GAC AAC ACT GTC AAG ATG GCC ACA CAG
Arg Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala Thr Gln>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

     1220     1230     1240     1250     1260
      *      *      *      *      *
GCC CCT GGT AGT CGG GGC TCC CTG GGT GAC TCT GAT GGC AAG CAT
Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys His>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

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Figure 14D

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      1270      1280      1290      1300
      *      *      *      *      *      *      *      *
GAG ACT GTG AAC CAG GAA GAG AAG AGT GGC CCA GGT GCA AGT GGG
Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>

      1310      1320      1330      1340      1350
      *      *      *      *      *      *      *      *
GGC AGC AGC GGG GAC CCT GAT GGC TTC TTC AAC TCC AAA GGC CCG
Gly Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys>
__c__c__c__ELK-L ECTODOMAIN 2 (NO SIGNAL)__c__c__c__>
                                     Gly Pro>
                                     __d__>

      1360      1370      1380      1390
      *      *      *      *      *      *      *      *
GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC
Gly>
__>
      Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys>
      __e__e__e__e__HUMAN IGG1 FC TAG__e__e__e__e__>

      1400      1410      1420      1430      1440
      *      *      *      *      *      *      *      *
CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro>
__e__e__e__e__HUMAN IGG1 FC TAG__e__e__e__e__>

      1450      1460      1470      1480
      *      *      *      *      *      *      *      *
CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val>
__e__e__e__e__HUMAN IGG1 FC TAG__e__e__e__e__>

      1490      1500      1510      1520      1530
      *      *      *      *      *      *      *      *
ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys>
__e__e__e__e__HUMAN IGG1 FC TAG__e__e__e__e__>

      1540      1550      1560      1570
      *      *      *      *      *      *      *      *
TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>
__e__e__e__e__HUMAN IGG1 FC TAG__e__e__e__e__>

      1580      1590      1600      1610      1620
      *      *      *      *      *      *      *      *
AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser>
__e__e__e__e__HUMAN IGG1 FC TAG__e__e__e__e__>

      1630      1640      1650      1660
      *      *      *      *      *      *      *      *
GTC CTC ACC GTC CTC CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr>
__e__e__e__e__HUMAN IGG1 FC TAG__e__e__e__e__>

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1270 1280 1290 1300 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660

[illegible]

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1670          1680          1690          1700          1710
*            *            *            *            *
AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys>
_e_e_e_e_e_____HUMAN IGG1 FC TAG_e_e_e_e_e_____>

          1720          1730          1740          1750
*            *            *            *            *
ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr>
_e_e_e_e_e_____HUMAN IGG1 FC TAG_e_e_e_e_e_____>

          1760          1770          1780          1790          1800
*            *            *            *            *
ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser>
_e_e_e_e_e_____HUMAN IGG1 FC TAG_e_e_e_e_e_____>

          1810          1820          1830          1840
*            *            *            *            *
CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val>
_e_e_e_e_e_____HUMAN IGG1 FC TAG_e_e_e_e_e_____>

          1850          1860          1870          1880          1890
*            *            *            *            *
GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr>
_e_e_e_e_e_____HUMAN IGG1 FC TAG_e_e_e_e_e_____>

          1900          1910          1920          1930
*            *            *            *            *
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>
_e_e_e_e_e_____HUMAN IGG1 FC TAG_e_e_e_e_e_____>

          1940          1950          1960          1970          1980
*            *            *            *            *
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser>
_e_e_e_e_e_____HUMAN IGG1 FC TAG_e_e_e_e_e_____>

          1990          2000          2010          2020
*            *            *            *            *
TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys>
_e_e_e_e_e_____HUMAN IGG1 FC TAG_e_e_e_e_e_____>

          2030          2040          2050
*            *            *            *            *
AGC CTC TCC CTG TCT CCG GGT AAA TGA
Ser Leu Ser Leu Ser Pro Gly Lys ***>
_e_e_e_____HUMAN IGG1 FC TAG_e_e_e_____>

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Figure 15A

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      10      20      30      40
      *      *      *      *
ATG GCC ATG GCC CGG TCC AGG AGG GAC TCT GTG TGG AAG TAC TGT
Met Ala Met Ala Arg Ser Arg Arg Asp Ser Val Trp Lys Tyr Cys>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE) __a__>

      50      60      70      80      90
      *      *      *      *      *
TGG GGA CTT TTG ATG GTT TTG TGC AGA ACT GCG ATC TCC AGA TCG
Trp Gly Leu Leu Met Val Leu Cys Arg Thr Ala Ile Ser Arg Ser>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE) __a__>

     100     110     120     130
      *      *      *      *      *
ATA GTT TTA GAG CCT ATC TAC TGG AAT TCC TCG AAC TCC AAA TTT
Ile Val Leu Glu Pro Ile Tyr Trp Asn Ser Ser Asn Ser Lys Phe>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE) __a__>

     140     150     160     170     180
      *      *      *      *      *
CTA CCC GGA CAA GGC CTG GTA CTA TAC CCA CAG ATA GGA GAC AAA
Leu Pro Gly Gln Gly Leu Val Leu Tyr Pro Gln Ile Gly Asp Lys>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE) __a__>

     190     200     210     220
      *      *      *      *      *
TTG GAT ATT ATT TGC CCC AAA GTG GAC TCT AAA ACT GTT GGC CAG
Leu GAT Ile Ile Cys Pro Lys Val Asp Ser Lys Thr Val Gly Gln>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE) __a__>

     230     240     250     260     270
      *      *      *      *      *
TAT GAA TAT TAT AAA GTT TAT ATG GTT GAT AAA GAC CAA GCA GAC
Tyr Gln Tyr Tyr Lys Val Tyr Met Val Asp Lys Asp Gln Ala Asp>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE) __a__>

     280     290     300     310
      *      *      *      *      *
AGA TGC ACA ATT AAG AAG GAG AAT ACC CCG CTG CTC AAC TGT GCC
Arg Cys Thr Ile Lys Lys Glu Asn Thr Pro Leu Leu Asn Cys Ala>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE) __a__>

     320     330     340     350     360
      *      *      *      *      *
AGA CCA GAC CAA GAT GTG AAA TTC ACC ATC AAG TTT CAA GAA TTC
Arg Pro Asp Gln Asp Val Lys Phe Thr Ile Lys Phe Gln Glu Phe>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE) __a__>

     370     380     390     400
      *      *      *      *
AGC CCT AAC CTC TGG GGT CTA GAA TTT CAG AAG AAC AAA GAT TAC
Ser Pro Asn Leu Trp Gly Leu Glu Phe Gln Lys Asn Lys Asp Tyr>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE) __a__>

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Figure 15B

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      410      420      430      440      450
      *      *      *      *      *
TAC ATT ATA TCT ACA TCA AAT GGG TCT TTG GAG GGC CTG GAT AAC
Tyr Ile Ile Ser Thr Ser Asn Gly Ser Leu Glu Gly Leu Asp Asn>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)__a__>

      460      470      480      490
      *      *      *      *      *
CAG GAG GGA GGG GTG TGC CAG ACA AGA GCC ATG AAG ATC CTC ATG
Gln Glu Gly Gly Val Cys Gln Thr Arg Ala Met Lys Ile Leu Met>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)__a__>

      500      510      520      530      540
      *      *      *      *      *
AAA GTT GGA CAA GAT GCA AGT TCT GCT GGA TCA GCC AGG AAT CAC
Lys Val Gly Gln Asp Ala Ser Ser Ala Gly Ser Ala Arg Asn His>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)__a__>

      550      560      570      580
      *      *      *      *      *
GGT CCA ACA AGA CGT CCA GAG CTA GAA GCT GGT ACA AAT GGG AGA
Gly Pro Thr Arg Arg Pro Glu Leu Glu Ala Gly Thr Asn Gly Arg>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)__a__>

      590      600      610      620      630
      *      *      *      *      *
AGT TCA ACA ACA AGT CCC TTT GTG AAG CCA AAT CCA GGT TCT AGC
Ser Ser Thr Thr Ser Pro Phe Val Lys Pro Asn Pro Gly Ser Ser>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)__a__>

      640      650      660      670
      *      *      *      *      *
ACC GAT GGC AAC AGC GCG GGG CAT TCC GGG AAC AAT CTC CTG GGG
Thr Asp Gly Asn Ser Ala Gly His Ser Gly Asn Asn Leu Leu Gly>
__a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)__a__>

      680      690      700      710      720
      *      *      *      *      *
GGC CCG GGA ATA GTT TTA GAG CCT ATC TAC TGG AAT TCC TCG AAC
Gly Pro Gly>
__b__b__>
      Ile Val Leu Glu Pro Ile Tyr Trp Asn Ser Ser Asn>
      __EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNA__>

      730      740      750      760
      *      *      *      *      *
TCC AAA TTT CTA CCC GGA CAA GGC CTG GTA CTA TAC CCA CAG ATA
Ser Lys Phe Leu Pro Gly Gln Gly Leu Val Leu Tyr Pro Gln Ile>
      __EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e__>

      770      780      790      800      810
      *      *      *      *      *
GGA GAC AAA TTG GAT ATT ATT TGC CCC AAA GTG GAC TCT AAA ACT
Gly Asp Lys Leu Asp Ile Ile Cys Pro Lys Val Asp Ser Lys Thr>
      __EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_c__>

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Figure 15C

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      820      830      840      850
      *      *      *      *      *
GTT GGC CAG TAT GAA TAT TAT AAA GTT TAT ATG GTT GAT AAA GAC
Val Gly Gln Tyr Glu Tyr Tyr Lys Val Tyr Met Val Asp Lys Asp>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

      860      870      880      890      900
      *      *      *      *      *
CAA GCA GAC AGA TGC ACA ATT AAG AAG GAG AAT ACC CCG CTG CTC
Gln Ala Asp Arg Cys Thr Ile Lys Lys Glu Asn Thr Pro Leu Leu>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

      910      920      930      940
      *      *      *      *      *
AAC TGT GCC AGA CCA GAC CAA GAT GTG AAA TTC ACC ATC AAG TTT
Asn Cys Ala Arg Pro Asp Gln Asp Val Lys Phe Thr Ile Lys Phe>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

      950      960      970      980      990
      *      *      *      *      *
CAA GAA TTC AGC CCT AAC CTC TGG GGT CTA GAA TTT CAG AAG AAC
Gln Glu Phe Ser Pro Asn Leu Trp Gly Leu Glu Phe Gln Lys Asn>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

      1000     1010     1020     1030
      *      *      *      *      *
AAA GAT TAC TAC ATT ATA TCT ACA TCA AAT GGG TCT TTG GAG GGC
Lys Asp Tyr Tyr Ile Ile Ser Thr Ser Asn Gly Ser Leu Glu Gly>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

      1040     1050     1060     1070     1080
      *      *      *      *      *
CTG GAT AAC CAG GAG GGA GGG GTG TGC CAG ACA AGA GCC ATG AAG
Leu Asp Asn Gln Glu Gly Gly Val Cys Gln Thr Arg Ala Met Lys>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

      1090     1100     1110     1120
      *      *      *      *      *
ATC CTC ATG AAA GTT GGA CAA GAT GCA AGT TCT GCT GGA TCA GCC
Ile Leu Met Lys Val Gly Gln Asp Ala Ser Ser Ala Gly Ser Ala>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

      1130     1140     1150     1160     1170
      *      *      *      *      *
AGG AAT CAC GGT CCA ACA AGA CGC CCA GAG CTA GAA GCT GGT ACA
Arg Asn His Gly Pro Thr Arg Arg Pro Glu Leu Glu Ala Gly Thr>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

      1180     1190     1200     1210
      *      *      *      *      *
AAT GGG AGA AGT TCA ACA ACA AGT CCC TTT GTG AAG CCA AAT CCA
Asn Gly Arg Ser Ser Thr Thr Ser Pro Phe Val Lys Pro Asn Pro>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

      1220     1230     1240     1250     1260
      *      *      *      *      *
GGT TCT AGC ACC GAT GGC AAC AGC GCG GGG CAT TCC GGG AAC AAT
Gly Ser Ser Thr Asp Gly Asn Ser Ala Gly His Ser Gly Asn Asn>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

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1270      1280      1290      1300
*         *         *         *         *         *         *         *
CTC CTG GGG G GC CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC
      Glu Pro Lys Ser Cys Asp Lys Thr His>
      ____c____HUMAN IGG1 FC TAG____c____c____>
      Gly Pro Gly>
      _d_d_d____>
Leu Leu Gly Xxx>
____e____e____e____>

1310      1320      1330      1340      1350
*         *         *         *         *         *         *         *
ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser>
____c____c____c____c____HUMAN IGG1 FC TAG____c____c____c____c____>

1360      1370      1380      1390
*         *         *         *         *         *         *         *
GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser>
____c____c____c____c____HUMAN IGG1 FC TAG____c____c____c____c____>

1400      1410      1420      1430      1440
*         *         *         *         *         *         *         *
CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu>
____c____c____c____c____HUMAN IGG1 FC TAG____c____c____c____c____>

1450      1460      1470      1480
*         *         *         *         *         *         *         *
GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val>
____c____c____c____c____HUMAN IGG1 FC TAG____c____c____c____c____>

1490      1500      1510      1520      1530
*         *         *         *         *         *         *         *
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr>
____c____c____c____c____HUMAN IGG1 FC TAG____c____c____c____c____>

1540      1550      1560      1570
*         *         *         *         *         *         *         *
TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu>
____c____c____c____c____HUMAN IGG1 FC TAG____c____c____c____c____>

1580      1590      1600      1610      1620
*         *         *         *         *         *         *         *
AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>
____c____c____c____c____HUMAN IGG1 FC TAG____c____c____c____c____>

1630      1640      1650      1660
*         *         *         *         *         *         *         *
GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg>
____c____c____c____c____HUMAN IGG1 FC TAG____c____c____c____c____>

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Figure 15E

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1670      1680      1690      1700      1710
*          *          *          *          *
GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr>
__c__c__c__c__HUMAN IGG1 FC TAG__c__c__c__c__c__>

      1720      1730      1740      1750
*          *          *          *          *
AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro>
__c__c__c__c__HUMAN IGG1 FC TAG__c__c__c__c__c__>

1760      1770      1780      1790      1800
*          *          *          *          *
AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn>
__c__c__c__c__HUMAN IGG1 FC TAG__c__c__c__c__c__>

      1810      1820      1830      1840
*          *          *          *          *
AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe>
__c__c__c__c__HUMAN IGG1 FC TAG__c__c__c__c__c__>

1850      1860      1870      1880      1890
*          *          *          *          *
TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln>
__c__c__c__c__HUMAN IGG1 FC TAG__c__c__c__c__c__>

      1900      1910      1920      1930
*          *          *          *          *
GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn>
__c__c__c__c__HUMAN IGG1 FC TAG__c__c__c__c__c__>

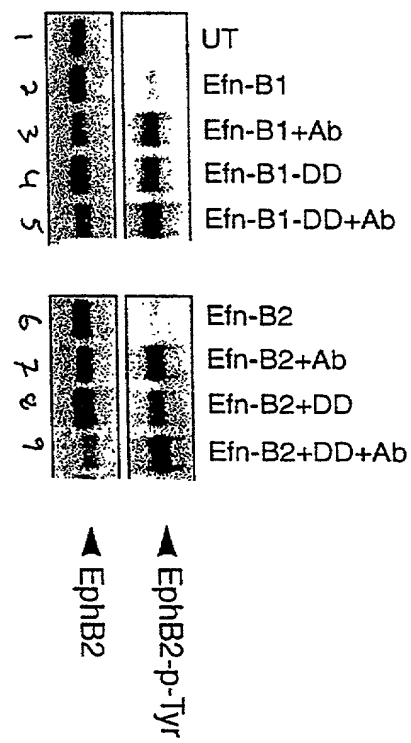
1940      1950      1960      1970
*          *          *          *          *
CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>
__c__c__c__c__HUMAN IGG1 FC TAG__c__c__c__c__c__>

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PCT/US99/30900

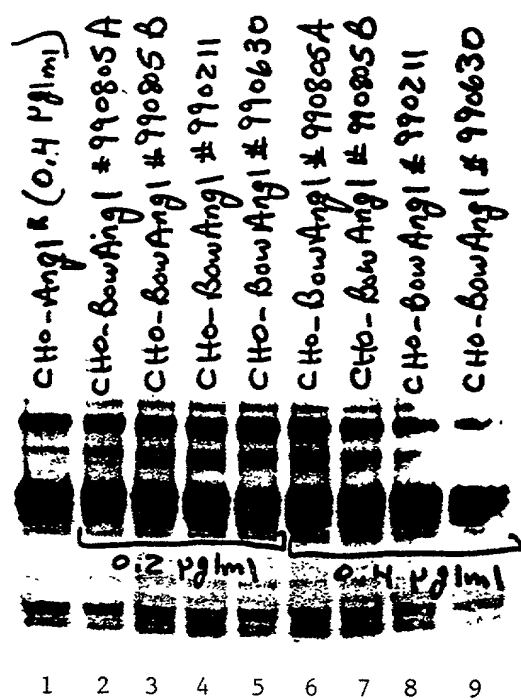
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Figure 16



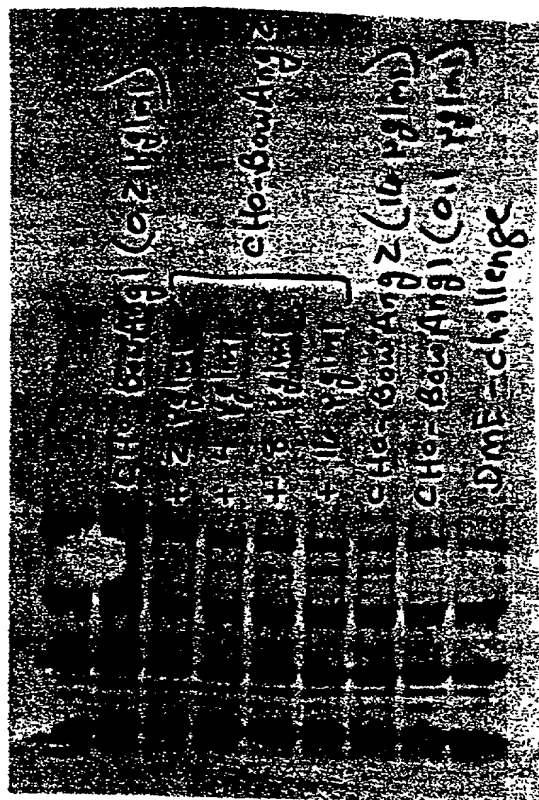
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Figure 17



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Figure 18



1 2 3 4 5 6 7 8 9

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled **METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS**, USSN 09/868,677 filed June 20, 2001, which is the national stage filing of International Application No. PCT/US99/30900, filed December 23, 1999.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to in the oath or declaration.

I acknowledge the duty to disclose information of which I am aware that is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PCT/US99/30900 filed December 23, 1999

I hereby claim the benefit under Title 35, United States Code, §119(e) and 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States Application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) that occurred between the filing date of the prior application and the national or PCT international filing date of this application:

USSN 60/113,387 filed December 23, 1998

3 And I hereby appoint Joseph M. Sorrentino (Registration No. 32,598), Gail M. Kempler (Registration No. 32,143), and Linda O. Palladino (Registration No. 45,636), each of them my attorneys and agent, each with full power of substitution and revocation, to prosecute this application, to make alterations and

Att. Docket No. REG 670A-US
National Stage Filing of
Int'l File No. PCT/US99/30900
Declaration and Power of Attorney

amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications that are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone calls, regarding this application to:

Linda O. Palladino
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Tel. (914-345-7400)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Int'l File No. PCT/US99/30900
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SEQUENCE LISTING

<110> Samuel Davis, Nicholas W. Gale, George D. Yancopoulos, and
Neil Stahl

<120> METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

<130> REG 670-A-US

<140> USSN 09/868,677

<141> 2001-10-01

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<151> 1999-12-23

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Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr
20 25 30

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Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn
35 40 45

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85 90 95

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100 105 110

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ggt gaa tat tgg ctg ggg aat gag ttt att ttt gcc att acc agt cag Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln 305 310 315 320	960
agg cag tac atg cta aga att gag tta atg gac tgg gaa ggg aac cga Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg 325 330 335	1008
gcc tat tca cag tat gac aga ttc cac ata gga aat gaa aag caa aac Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn 340 345 350	1056

tat agg ttg tat tta aaa ggt cac act ggg aca gca gga aaa cag agc Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser 355 360 365	1104
agc ctg atc tta cac ggt gct gat ttc agc act aaa gat gct gat aat Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn 370 375 380	1152
gac aac tgt atg tgc aaa tgt gcc ctc atg tta aca gga gga tgg tgg Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp 385 390 395 400	1200
ttt gat gct tgt ggc ccc tcc aat cta aat gga atg ttc tat act gcg Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala 405 410 415	1248
gga caa aac cat gga aaa ctg aat ggg ata aag tgg cac tac ttc aaa Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys 420 425 430	1296
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acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys 545 550 555 560	1680
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gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser 580 585 590	1776
cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa	1824

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Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu									
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Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys									
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Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys									
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Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu									
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Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys									
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Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys									
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Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser									
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Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr	
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acg tta aca ttc cct aat tct aca gaa gag atc aag gcc tac tgt gac	144
Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp	
35 40 45	
atg gaa gct gga gga ggc ggg tgg aca att att cag cga cgt gag gat	192
Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp	
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ggc agc gtt gat ttt cag agg act tgg aaa gaa tat aaa gtg gga ttt	240
Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe	
65 70 75 80	
ggg aac cct tca gga gaa tat tgg ctg gga aat gag ttt gtt tcg caa	288
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln	
85 90 95	
ctg act aat cag caa cgc tat gtg ctt aaa ata cac ctt aaa gac tgg	336
Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp	
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gaa ggg aat gag gct tac tca ttg tat gaa cat ttc tat ctc tca agt	384
Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser	
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Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala	
130 135 140	
ggc aaa ata agc agc atc agc caa cca gga aat gat ttt agc aca aag	480
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145 150 155 160	
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Asp Gly Asp Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr	
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Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met	
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Tyr Tyr Pro Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp	
195 200 205	
tac tac tgg aaa ggc tca ggc tat tcg ctc aag gcc aca acc atg atg	672
Tyr Tyr Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met	
210 215 220	

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tca gga gaa tat tgg ctg gga aat gag ttt gtt tcg caa ctg act aat Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn 305 310 315 320	960
cag caa cgc tat gtg ctt aaa ata cac ctt aaa gac tgg gaa ggg aat Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn 325 330 335	1008
gag gct tac tca ttg tat gaa cat ttc tat ctc tca agt gaa gaa ctc Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu 340 345 350	1056
aat tat agg att cac ctt aaa gga ctt aca ggg aca gcc ggc aaa ata Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile 355 360 365	1104
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aaa ggc tca ggc tat tcg ctc aag gcc aca acc atg atg atc cga cca Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro 435 440 445	1344
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ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro 485 490 495	1488
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aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr 515 520 525	1584
aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val 530 535 540	1632
ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys 545 550 555 560	1680
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aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro 580 585 590	1776
tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val 595 600 605	1824
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Leu	Thr	Asn	Gln	Gln	Arg	Tyr	Val	Leu	Lys	Ile	His	Leu	Lys	Asp	Trp
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Tyr	Tyr	Pro	Gln	Arg	Gln	Asn	Thr	Asn	Lys	Phe	Asn	Gly	Ile	Lys	Trp
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Glu	Val	Phe	Lys	Ser	Gly	His	Thr	Thr	Asn	Gly	Ile	Tyr	Thr	Leu	Thr
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Phe	Pro	Asn	Ser	Thr	Glu	Glu	Ile	Lys	Ala	Tyr	Cys	Asp	Met	Glu	Ala
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Gln	Gln	Arg	Tyr	Val	Leu	Lys	Ile	His	Leu	Lys	Asp	Trp	Glu	Gly	Asn
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Glu	Ala	Tyr	Ser	Leu	Tyr	Glu	His	Phe</							

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
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 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
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 500 505 510
 Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
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 Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
 565 570 575
 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
 580 585 590
 Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
 595 600 605
 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
 610 615 620
 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
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 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
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Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn	
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atg gat gtc aat ggg gga ggt tgg act gta ata caa cat cgt gaa gat	192
Met Asp Val Asn Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp	
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gga agt cta gat ttc caa aga ggc tgg aag gaa tat aaa atg ggt ttt	240
Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe	
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Ile	Thr	Ser	Gln	Arg	Gln	Tyr	Met	Leu	Arg	Ile	Glu	Leu	Met	Asp	Trp	
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Glu	Lys	Gln	Asn	Tyr	Arg	Leu	Tyr	Leu	Lys	Gly	His	Thr	Gly	Thr	Ala	
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Gly	Gly	Trp	Trp	Phe	Asp	Ala	Cys	Gly	Pro	Ser	Asn	Leu	Asn	Gly	Met	
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Phe	Tyr	Thr	Ala	Gly	Gln	Asn	His	Gly	Lys	Leu	Asn	Gly	Ile	Lys	Trp	
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His	Tyr	Phe	Lys	Gly	Pro	Ser	Tyr	Ser	Leu	Arg	Ser	Thr	Thr	Met	Met	
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Ile	Arg	Pro	Leu	Asp	Phe	Gly	Pro	Gly	Glu	Pro	Lys	Ser	Cys	Asp	Lys	
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Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	
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tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	tcc	816
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	
			260					265					270			
cgg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	864
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	
		275					280					285				
cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	912
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	
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Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr
580 585 590

agg ttg tat tta aaa ggt cac act ggg aca gca gga aaa cag agc agc 1824
Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser
595 600 605

ctg atc tta cac ggt gct gat ttc agc act aaa gat gct gat aat gac 1872
Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp
610 615 620

aac tgt atg tgc aaa tgt gcc ctc atg tta aca gga gga tgg tgg ttt 1920
Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe
625 630 635 640

gat gct tgt ggc ccc tcc aat cta aat gga atg ttc tat act gcg gga 1968
Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly
645 650 655

caa aac cat gga aaa ctg aat ggg ata aag tgg cac tac ttc aaa ggg 2016
Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly
660 665 670

cca agt tac tcc tta cgt tcc aca act atg atg att cga cct tta gat 2064
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ttt 2067

<210> 6
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<212> PRT
<213> Homo sapiens

<400> 6

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35 40 45
Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp
50 55 60
Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe
65 70 75 80
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala
85 90 95
Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp
100 105 110
Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn
115 120 125
Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala
130 135 140
Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys
145 150 155 160
Asp Ala Asp Asn Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr
165 170 175
Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met
180 185 190

Phe	Tyr	Thr	Ala	Gly	Gln	Asn	His	Gly	Lys	Leu	Asn	Gly	Ile	Lys	Trp
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His	Tyr	Phe	Lys	Gly	Pro	Ser	Tyr	Ser	Leu	Arg	Ser	Thr	Thr	Met	Met
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Ile	Arg	Pro	Leu	Asp	Phe	Gly	Pro	Gly	Glu	Pro	Lys	Ser	Cys	Asp	Lys
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Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro
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Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
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Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp
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Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
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Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu
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Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys
			340					345					350		
Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr
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Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr
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Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu
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Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu
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Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys
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Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu
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Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly
			450			455					460				
Lys	Gly	Gly	Gly	Gly	Ser	Gly	Ala	Pro	Phe	Arg	Asp	Cys	Ala	Asp	Val
465					470					475					480
Tyr	Gln	Ala	Gly	Phe	Asn	Lys	Ser	Gly	Ile	Tyr	Thr	Ile	Tyr	Ile	Asn
				485					490					495	
Asn	Met	Pro	Glu	Pro	Lys	Lys	Val	Phe	Cys	Asn	Met	Asp	Val	Asn	Gly
			500					505					510		
Gly	Gly	Trp	Thr	Val	Ile	Gln	His	Arg	Glu	Asp	Gly	Ser	Leu	Asp	Phe
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Gln	Arg	Gly	Trp	Lys	Glu	Tyr	Lys	Met	Gly	Phe	Gly	Asn	Pro	Ser	Gly
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675

680

685

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 <211> 2070
 <212> DNA
 <213> Homo sapiens

<220>

<221> CDS
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Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr	
20 25 30	
acg tta aca ttc cct aat tct aca gaa gag atc aag gcc tac tgt gac	144
Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp	
35 40 45	
atg gaa gct gga gga ggc ggg tgg aca att att cag cga cgt gag gat	192
Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp	
50 55 60	
ggc agc gtt gat ttt cag agg act tgg aaa gaa tat aaa gtg gga ttt	240
Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe	
65 70 75 80	
ggg aac cct tca gga gaa tat tgg ctg gga aat gag ttt gtt tcg caa	288
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln	
85 90 95	
ctg act aat cag caa cgc tat gtg ctt aaa ata cac ctt aaa gac tgg	336
Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp	
100 105 110	
gaa ggg aat gag gct tac tca ttg tat gaa cat ttc tat ctc tca agt	384
Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser	
115 120 125	
gaa gaa ctc aat tat agg att cac ctt aaa gga ctt aca ggg aca gcc	432
Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala	
130 135 140	
ggc aaa ata agc agc atc agc caa cca gga aat gat ttt agc aca aag	480
Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys	
145 150 155 160	
gat gga gac aac gac aaa tgt att tgc aaa tgt tca caa atg cta aca	528
Asp Gly Asp Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr	
165 170 175	
gga ggc tgg tgg ttt gat gca tgt ggt cct tcc aac ttg aac gga atg	576
Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met	
180 185 190	

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Tyr Tyr Pro Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp	
195 200 205	
tac tac tgg aaa ggc tca ggc tat tgc ctc aag gcc aca acc atg atg	672
Tyr Tyr Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met	
210 215 220	
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Ile Arg Pro Ala Asp Phe Gly Gly Pro Gly Glu Pro Lys Ser Cys Asp	
225 230 235 240	
aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga	768
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly	
245 250 255	
ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc	816
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile	
260 265 270	
tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa	864
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu	
275 280 285	
gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat	912
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His	
290 295 300	
aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt	960
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg	
305 310 315 320	
gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag	1008
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys	
325 330 335	
gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag	1056
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu	
340 345 350	
aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac	1104
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr	
355 360 365	
acc ctg ccc cca tcc ccg gat gag ctg acc aag aac cag gtc agc ctg	1152
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu	
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acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg	1200
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp	
385 390 395 400	
gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg	1248
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val	
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ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac	1296
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp	
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aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat	1344

Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His		
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gag	gct	ctg	cac	aac	cac	tac	acg	cag	aag	agc	ctc	tcc	ctg	tct	ccg	1392	
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	450					455					460						
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Gly	Lys	Gly	Gly	Gly	Gly	Ser	Gly	Ala	Pro	Arg	Asp	Cys	Ala	Glu	Val		
	465				470					475				480			
ttc	aaa	tca	gga	cac	acc	aca	aat	ggc	atc	tac	acg	tta	aca	ttc	cct	1488	
Phe	Lys	Ser	Gly	His	Thr	Thr	Asn	Gly	Ile	Tyr	Thr	Leu	Thr	Phe	Pro		
				485					490					495			
aat	tct	aca	gaa	gag	atc	aag	gcc	tac	tgt	gac	atg	gaa	gct	gga	gga	1536	
Asn	Ser	Thr	Glu	Glu	Ile	Lys	Ala	Tyr	Cys	Asp	Met	Glu	Ala	Gly	Gly		
			500					505					510				
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Gly	Gly	Trp	Thr	Ile	Ile	Gln	Arg	Arg	Glu	Asp	Gly	Ser	Val	Asp	Phe		
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cag	agg	act	tgg	aaa	gaa	tat	aaa	gtg	gga	ttt	ggt	aac	cct	tca	gga	1632	
Gln	Arg	Thr	Trp	Lys	Glu	Tyr	Lys	Val	Gly	Phe	Gly	Asn	Pro	Ser	Gly		
		530				535						540					
gaa	tat	tgg	ctg	gga	aat	gag	ttt	gtt	tgc	caa	ctg	act	aat	cag	caa	1680	
Glu	Tyr	Trp	Leu	Gly	Asn	Glu	Phe	Val	Ser	Gln	Leu	Thr	Asn	Gln	Gln		
	545				550					555					560		
cgc	tat	gtg	ctt	aaa	ata	cac	ctt	aaa	gac	tgg	gaa	ggg	aat	gag	gct	1728	
Arg	Tyr	Val	Leu	Lys	Ile	His	Leu	Lys	Asp	Trp	Glu	Gly	Asn	Glu	Ala		
				565					570					575			
tac	tca	ttg	tat	gaa	cat	ttc	tat	ctc	tca	agt	gaa	gaa	ctc	aat	tat	1776	
Tyr	Ser	Leu	Tyr	Glu	His	Phe	Tyr	Leu	Ser	Ser	Glu	Glu	Leu	Asn	Tyr		
			580					585					590				
agg	att	cac	ctt	aaa	gga	ctt	aca	ggg	aca	gcc	ggc	aaa	ata	agc	agc	1824	
Arg	Ile	His	Leu	Lys	Gly	Leu	Thr	Gly	Thr	Ala	Gly	Lys	Ile	Ser	Ser		
		595					600					605					
atc	agc	caa	cca	gga	aat	gat	ttt	agc	aca	aag	gat	gga	gac	aac	gac	1872	
Ile	Ser	Gln	Pro	Gly	Asn	Asp	Phe	Ser	Thr	Lys	Asp	Gly	Asp	Asn	Asp		
	610					615					620						
aaa	tgt	att	tgc	aaa	tgt	tca	caa	atg	cta	aca	gga	ggc	tgg	tgg	ttt	1920	
Lys	Cys	Ile	Cys	Lys	Cys	Ser	Gln	Met	Leu	Thr	Gly	Gly	Trp	Trp	Phe		
	625				630					635					640		
gat	gca	tgt	ggt	cct	tcc	aac	ttg	aac	gga	atg	tac	tat	cca	cag	agg	1968	
Asp	Ala	Cys	Gly	Pro	Ser	Asn	Leu	Asn	Gly	Met	Tyr	Tyr	Pro	Gln	Arg		
				645					650					655			
cag	aac	aca	aat	aag	ttc	aac	ggc	att	aaa	tgg	tac	tac	tgg	aaa	ggc	2016	
Gln	Asn	Thr	Asn	Lys	Phe	Asn	Gly	Ile	Lys	Trp	Tyr	Tyr	Trp	Lys	Gly		
			660					665					670				
tca	ggc	tat	tgc	ctc	aag	gcc	aca	acc	atg	atg	atc	cga	cca	gca	gat	2064	
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675

680

685

ttc tga
Phe

2070

<210> 8
<211> 689
<212> PRT
<213> Homo sapiens

<400> 8

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Thr	Leu	Thr	Phe	Pro	Asn	Ser	Thr	Glu	Glu	Ile	Lys	Ala	Tyr	Cys	Asp
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Met	Glu	Ala	Gly	Gly	Gly	Gly	Trp	Thr	Ile	Ile	Gln	Arg	Arg	Glu	Asp
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Gly	Asn	Pro	Ser	Gly	Glu	Tyr	Trp	Leu	Gly	Asn	Glu	Phe	Val	Ser	Gln
				85				90					95		
Leu	Thr	Asn	Gln	Arg	Tyr	Val	Leu	Lys	Ile	His	Leu	Lys	Asp	Trp	
		100					105					110			
Glu	Gly	Asn	Glu	Ala	Tyr	Ser	Leu	Tyr	Glu	His	Phe	Tyr	Leu	Ser	Ser
		115					120					125			
Glu	Glu	Leu	Asn	Tyr	Arg	Ile	His	Leu	Lys	Gly	Leu	Thr	Gly	Thr	Ala
	130					135					140				
Gly	Lys	Ile	Ser	Ser	Ile	Ser	Gln	Pro	Gly	Asn	Asp	Phe	Ser	Thr	Lys
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Gly	Gly	Trp	Trp	Phe	Asp	Ala	Cys	Gly	Pro	Ser	Asn	Leu	Asn	Gly	Met
		180						185					190		
Tyr	Tyr	Pro	Gln	Arg	Gln	Asn	Thr	Asn	Lys	Phe	Asn	Gly	Ile	Lys	Trp
		195					200					205			
Tyr	Tyr	Trp	Lys	Gly	Ser	Gly	Tyr	Ser	Leu	Lys	Ala	Thr	Thr	Met	Met
	210					215					220				
Ile	Arg	Pro	Ala	Asp	Phe	Gly	Gly	Pro	Gly	Glu	Pro	Lys	Ser	Cys	Asp
225					230					235				240	
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly
				245					250					255	
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile
		260						265					270		
Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu
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Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His
	290					295					300				
Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg
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Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu
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	355						360					365			
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	370					375					380				

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
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 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
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 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
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 465 470 475 480
 Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro
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 Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly
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 Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly
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 Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln
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 Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala
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 Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr
 580 585 590
 Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser
 595 600 605
 Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp
 610 615 620
 Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe
 625 630 635 640
 Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg
 645 650 655
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<220>
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Val Val Trp Ala Leu Cys Arg Leu Ala Thr Pro Leu Ala Lys Asn Leu	
20 25 30	
gag ccc gta tcc tgg agc tcc ctc aac ccc aag ttc ctg agt ggg aag	144
Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu Ser Gly Lys	

35					40					45						
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Gly	Leu	Val	Ile	Tyr	Pro	Lys	Ile	Gly	Asp	Lys	Leu	Asp	Ile	Ile	Cys	
50					55					60						
ccc	cga	gca	gaa	gca	ggg	cgg	ccc	tat	gag	tac	tac	aag	ctg	tac	ctg	240
Pro	Arg	Ala	Glu	Ala	Gly	Arg	Pro	Tyr	Glu	Tyr	Tyr	Lys	Leu	Tyr	Leu	
65					70					75					80	
gtg	cgg	cct	gag	cag	gca	gct	gcc	tgt	agc	aca	gtt	ctc	gac	ccc	aac	288
Val	Arg	Pro	Glu	Gln	Ala	Ala	Ala	Cys	Ser	Thr	Val	Leu	Asp	Pro	Asn	
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Val	Leu	Val	Thr	Cys	Asn	Arg	Pro	Glu	Gln	Glu	Ile	Arg	Phe	Thr	Ile	
100					105					110						
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Lys	Phe	Gln	Glu	Phe	Ser	Pro	Asn	Tyr	Met	Gly	Leu	Glu	Phe	Lys	Lys	
115					120					125						
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His	His	Asp	Tyr	Tyr	Ile	Thr	Ser	Thr	Ser	Asn	Gly	Ser	Leu	Glu	Gly	
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Leu	Glu	Asn	Arg	Glu	Gly	Gly	Val	Cys	Arg	Thr	Arg	Thr	Met	Lys	Ile	
145					150					155					160	
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Ile	Met	Lys	Val	Gly	Gln	Asp	Pro	Asn	Ala	Val	Thr	Pro	Glu	Gln	Leu	
165					170					175						
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Thr	Thr	Ser	Arg	Pro	Ser	Lys	Glu	Ala	Asp	Asn	Thr	Val	Lys	Met	Ala	
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Thr	Gln	Ala	Pro	Gly	Ser	Arg	Gly	Ser	Leu	Gly	Asp	Ser	Asp	Gly	Lys	
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His	Glu	Thr	Val	Asn	Gln	Glu	Glu	Lys	Ser	Gly	Pro	Gly	Ala	Ser	Gly	
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Gly	Ser	Ser	Gly	Asp	Pro	Asp	Gly	Phe	Phe	Asn	Ser	Lys	Gly	Pro	Gly	
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Lys	Asn	Leu	Glu	Pro	Val	Ser	Trp	Ser	Ser	Leu	Asn	Pro	Lys	Phe	Leu	
245					250					255						
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Ser	Gly	Lys	Gly	Leu	Val	Ile	Tyr	Pro	Lys	Ile	Gly	Asp	Lys	Leu	Asp	
260					265					270						
atc	atc	tgc	ccc	cga	gca	gaa	gca	ggg	cgg	ccc	tat	gag	tac	tac	aag	864

ctg Leu	tac Tyr	ctg Leu	gtg Val	cgg Arg	cct Pro	gag Glu	cag Gln	gca Ala	gct Ala	gcc Ala	tgt Cys	agc Ser	aca Thr	gtt Val	ctc Leu	912
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cca Pro	aaa Lys	ccc Pro	aag Lys	gac Asp	acc Thr	ctc Leu	atg Met	atc Ile	tcc Ser	cgg Arg	acc Thr	cct Pro	gag Glu	gtc Val	aca Thr	1488
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Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val	
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ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc	1680
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser	
545 550 555 560	
aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa	1728
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys	
565 570 575	
ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat	1776
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp	
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gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc	1824
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe	
595 600 605	
tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag	1872
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu	
610 615 620	
aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc	1920
Asn Asn Tyr Lys Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe	
625 630 635 640	
ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg	1968
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly	
645 650 655	
aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac	2016
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr	
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Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu Ser Gly Lys	
35 40 45	
Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu Asp Ile Ile Cys	
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Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr Lys Leu Tyr Leu	
65 70 75 80	
Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val Leu Asp Pro Asn	
85 90 95	
Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile Arg Phe Thr Ile	
100 105 110	

Lys	Phe	Gln	Glu	Phe	Ser	Pro	Asn	Tyr	Met	Gly	Leu	Glu	Phe	Lys	Lys
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Leu	Glu	Asn	Arg	Glu	Gly	Gly	Val	Cys	Arg	Thr	Arg	Thr	Met	Lys	Ile
		145				150				155					160
Ile	Met	Lys	Val	Gly	Gln	Asp	Pro	Asn	Ala	Val	Thr	Pro	Glu	Gln	Leu
				165					170					175	
Thr	Thr	Ser	Arg	Pro	Ser	Lys	Glu	Ala	Asp	Asn	Thr	Val	Lys	Met	Ala
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Thr	Gln	Ala	Pro	Gly	Ser	Arg	Gly	Ser	Leu	Gly	Asp	Ser	Asp	Gly	Lys
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His	Glu	Thr	Val	Asn	Gln	Glu	Glu	Lys	Ser	Gly	Pro	Gly	Ala	Ser	Gly
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Gly	Ser	Ser	Gly	Asp	Pro	Asp	Gly	Phe	Phe	Asn	Ser	Lys	Gly	Pro	Gly
		225			230					235					240
Lys	Asn	Leu	Glu	Pro	Val	Ser	Trp	Ser	Ser	Leu	Asn	Pro	Lys	Phe	Leu
				245					250					255	
Ser	Gly	Lys	Gly	Leu	Val	Ile	Tyr	Pro	Lys	Ile	Gly	Asp	Lys	Leu	Asp
			260						265				270		
Ile	Ile	Cys	Pro	Arg	Ala	Glu	Ala	Gly	Arg	Pro	Tyr	Glu	Tyr	Tyr	Lys
		275					280					285			
Leu	Tyr	Leu	Val	Arg	Pro	Glu	Gln	Ala	Ala	Ala	Cys	Ser	Thr	Val	Leu
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Asp	Pro	Asn	Val	Leu	Val	Thr	Cys	Asn	Arg	Pro	Glu	Gln	Glu	Ile	Arg
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Phe	Thr	Ile	Lys	Phe	Gln	Glu	Phe	Ser	Pro	Asn	Tyr	Met	Gly	Leu	Glu
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Phe	Lys	Lys	His	His	Asp	Tyr	Tyr	Ile	Thr	Ser	Thr	Ser	Asn	Gly	Ser
			340					345					350		
Leu	Glu	Gly	Leu	Glu	Asn	Arg	Glu	Gly	Gly	Val	Cys	Arg	Thr	Arg	Thr
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Met	Lys	Ile	Ile	Met	Lys	Val	Gly	Gln	Asp	Pro	Asn	Ala	Val	Thr	Pro
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Glu	Gln	Leu	Thr	Thr	Ser	Arg	Pro	Ser	Lys	Glu	Ala	Asp	Asn	Thr	Val
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Lys	Met	Ala	Thr	Gln	Ala	Pro	Gly	Ser	Arg	Gly	Ser	Leu	Gly	Asp	Ser
				405					410					415	
Asp	Gly	Lys	His	Glu	Thr	Val	Asn	Gln	Glu	Glu	Lys	Ser	Gly	Pro	Gly
			420					425					430		
Ala	Ser	Gly	Gly	Ser	Ser	Gly	Asp	Pro	Asp	Gly	Phe	Phe	Asn	Ser	Lys
		435					440					445			
Gly	Pro	Gly	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro
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Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro
		465				470				475					480
Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr
				485					490					495	
Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn
			500					505					510		
Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg
		515						520					525		
Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val
		530				535					540				
Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser
		545				550				555					560
Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys
				565					570					575	
Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp
			580					585					590		
Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe

595 600 605
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
610 615 620
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
625 630 635 640
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
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Gly Leu Leu Met Val Leu Cys Arg Thr Ala Ile Ser Arg Ser Ile Val
20 25 30
tta gag cct atc tac tgg aat tcc tcg aac tcc aaa ttt cta ccc gga 144
Leu Glu Pro Ile Tyr Trp Asn Ser Ser Asn Ser Lys Phe Leu Pro Gly
35 40 45
caa ggc ctg gta cta tac cca cag ata gga gac aaa ttg gat att att 192
Gln Gly Leu Val Leu Tyr Pro Gln Ile Gly Asp Lys Leu Asp Ile Ile
50 55 60
tgc ccc aaa gtg gac tct aaa act gtt ggc cag tat gaa tat tat aaa 240
Cys Pro Lys Val Asp Ser Lys Thr Val Gly Gln Tyr Glu Tyr Tyr Lys
65 70 75 80
gtt tat atg gtt gat aaa gac caa gca gac aga tgc aca att aag aag 288
Val Tyr Met Val Asp Lys Asp Gln Ala Asp Arg Cys Thr Ile Lys Lys
85 90 95
gag aat acc ccg ctg ctc aac tgt gcc aga cca gac caa gat gtg aaa 336
Glu Asn Thr Pro Leu Leu Asn Cys Ala Arg Pro Asp Gln Asp Val Lys
100 105 110
ttc acc atc aag ttt caa gaa ttc agc cct aac ctc tgg ggt cta gaa 384
Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro Asn Leu Trp Gly Leu Glu
115 120 125
ttt cag aag aac aaa gat tac tac att ata tct aca tca aat ggg tct 432
Phe Gln Lys Asn Lys Asp Tyr Tyr Ile Ile Ser Thr Ser Asn Gly Ser
130 135 140
ttg gag ggc ctg gat aac cag gag gga ggg gtg tgc cag aca aga gcc 480

Leu Glu Gly Leu Asp Asn Gln Glu Gly Gly Val Cys Gln Thr Arg Ala	
145 150 155 160	
atg aag atc ctc atg aaa gtt gga caa gat gca agt tct gct gga tca	528
Met Lys Ile Leu Met Lys Val Gly Gln Asp Ala Ser Ser Ala Gly Ser	
165 170 175	
gcc agg aat cac ggt cca aca aga cgt cca gag cta gaa gct ggt aca	576
Ala Arg Asn His Gly Pro Thr Arg Arg Pro Glu Leu Glu Ala Gly Thr	
180 185 190	
aat ggg aga agt tca aca aca agt ccc ttt gtg aag cca aat cca ggt	624
Asn Gly Arg Ser Ser Thr Thr Ser Pro Phe Val Lys Pro Asn Pro Gly	
195 200 205	
tct agc acc gat ggc aac agc gcg ggg cat tcc ggg aac aat ctc ctg	672
Ser Ser Thr Asp Gly Asn Ser Ala Gly His Ser Gly Asn Asn Leu Leu	
210 215 220	
ggg ggc ccg gga ata gtt tta gag cct atc tac tgg aat tcc tcg aac	720
Gly Gly Pro Gly Ile Val Leu Glu Pro Ile Tyr Trp Asn Ser Ser Asn	
225 230 235 240	
tcc aaa ttt cta ccc gga caa ggc ctg gta cta tac cca cag ata gga	768
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Asp Lys Leu Asp Ile Ile Cys Pro Lys Val Asp Ser Lys Thr Val Gly	
260 265 270	
cag tat gaa tat tat aaa gtt tat atg gtt gat aaa gac caa gca gac	864
Gln Tyr Glu Tyr Tyr Lys Val Tyr Met Val Asp Lys Asp Gln Ala Asp	
275 280 285	
aga tgc aca att aag aag gag aat acc ccg ctg ctc aac tgt gcc aga	912
Arg Cys Thr Ile Lys Lys Glu Asn Thr Pro Leu Leu Asn Cys Ala Arg	
290 295 300	
cca gac caa gat gtg aaa ttc acc atc aag ttt caa gaa ttc agc cct	960
Pro Asp Gln Asp Val Lys Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro	
305 310 315 320	
aac ctc tgg ggt cta gaa ttt cag aag aac aaa gat tac tac att ata	1008
Asn Leu Trp Gly Leu Glu Phe Gln Lys Asn Lys Asp Tyr Tyr Ile Ile	
325 330 335	
tct aca tca aat ggg tct ttg gag ggc ctg gat aac cag gag gga ggg	1056
Ser Thr Ser Asn Gly Ser Leu Glu Gly Leu Asp Asn Gln Glu Gly Gly	
340 345 350	
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Val Cys Gln Thr Arg Ala Met Lys Ile Leu Met Lys Val Gly Gln Asp	
355 360 365	
gca agt tct gct gga tca gcc agg aat cac ggt cca aca aga cgc cca	1152
Ala Ser Ser Ala Gly Ser Ala Arg Asn His Gly Pro Thr Arg Arg Pro	
370 375 380	
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Glu Leu Glu Ala Gly Thr Asn Gly Arg Ser Ser Thr Thr Ser Pro Phe	

0986667 "100101"

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Val Lys Pro Asn Pro Gly Ser Ser Thr Asp Gly Asn Ser Ala Gly His				
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tcc ggg aac aat ctc ctg ggg ggc ccg ggc gag ccc aaa tct tgt gac				1296
Ser Gly Asn Asn Leu Leu Gly Gly Pro Gly Glu Pro Lys Ser Cys Asp				
	420	425	430	
aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga				1344
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly				
	435	440	445	
ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc				1392
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile				
	450	455	460	
tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa				1440
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu				
	465	470	475	480
gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat				1488
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His				
	485	490	495	
aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt				1536
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg				
	500	505	510	
gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag				1584
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys				
	515	520	525	
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Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu				
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aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac				1680
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr				
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acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg				1728
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu				
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acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg				1776
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp				
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Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val				
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Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp				
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Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His				
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1968

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 Gly Lys

1977

<210> 12
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<220>

<223> Homo Sapiens Fusion Protein

<400> 12

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Gln	Gly	Leu	Val	Leu	Tyr	Pro	Gln	Ile	Gly	Asp	Lys	Leu	Asp	Ile	Ile
	50					55				60					
Cys	Pro	Lys	Val	Asp	Ser	Lys	Thr	Val	Gly	Gln	Tyr	Glu	Tyr	Tyr	Lys
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Val	Tyr	Met	Val	Asp	Lys	Asp	Gln	Ala	Asp	Arg	Cys	Thr	Ile	Lys	Lys
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Glu	Asn	Thr	Pro	Leu	Leu	Asn	Cys	Ala	Arg	Pro	Asp	Gln	Asp	Val	Lys
			100					105					110		
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